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ORIGINAL ARTICLES

RELATION OF DIFFERENTIATION AND LYMPHOCYTIC INFILTRATION TO POSTOPERATIVE LONGEVITY IN GASTRIC CARCINOMA*

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IN 1912 one of us (MacCarty)** published an article reporting the relation of regional lymphatic glandular involvement to postoperative longevity in gastric carcinoma. The series of specimens which was studied consisted of 200 resected portions of the stomach. The location and relative size of the gastric lymphatic glands were indicated on diagrams and each gland was examined microscopically for carcinoma. The specimens were divided into three groups,

Fig. 1.—No glandular involvement.

Fig. 2.—Involvement of some glands.

Fig. 3.—Involvement of all glands.

i. e., Group I, specimens with no glandular involvement; Group II, specimens with some glandular involvement; and Group III, specimens with all of the glands involved (Figs. 1, 2, 3).

The investigation of that series revealed the following facts:

1. Carcinomatous ulcers in the stomach varied from 1 centimeter to 14 centimeters in diameter and from 1 centimeter to 5 centimeters in depth.

*Read before the American Association for Cancer Research, New York, April, 1920.

**MacCarty, Wm. Carpenter, and Blackford, J. M.: Involvement of Regional Lymphatic Glands in Carcinoma of the Stomach, Ann. Surg., June, 1912.

2. The average ages in relation to glandular involvement at operation were: Group I, 50.8 years; Group II, 51.7 years; Group III, 46 years.

3. The duration of "dyspepsia" in the three groups, respectively, was 8.5 years, 8+years, and 8+ years.

4. The average duration of symptoms was 17.2 months, 12.5 months and 17.4 months.

5. The average loss of weight was 25 pounds, 28.4 pounds and 42.5 pounds.

6. The hospital mortality was 7.6 per cent, 11.6 per cent, and 18.7 per cent.

7. The size of regional lymphatic glands in gastric carcinoma bore no apparent relation to the size of the primary lesion in the stomach.

8. The size of a regional lymphatic gland was no criterion of the presence or absence of carcinoma.

9. Gross diagnosis of the presence or absence of carcinoma in lymphatic glands was of no value excepting in advanced carcinoma of the glands.

10. The duration of clinical symptoms bore no apparent relation to the size, and extent of involvement in lymphatic glands.

11. The average age and sex, at operation, bore no direct relation to the glandular involvement.

12. The average loss of weight increased with the increase in amount of glandular involvement.

13. The immediate hospital postoperative mortality was in direct proportion to the amount of glandular involvement.

14. The subsequent, or posthospital, mortality was in direct proportion to the amount of glandular involvement.

	GROUP I	GROUP II	GROUP III
15. Average age of patients	51.3 yrs.	49.7 yrs.	45.8 yrs.
Youngest	30 "	29 "	30 "
Oldest	72 "	68 "	66 "

16. Average length of life of the whole series in different decades	29-40	40-50	50-60	60-72
	2.7 yrs.	2.5 yrs.	2.3 yrs.	3+ yrs.

At the expiration of eight years after the first investigation, we were able to determine all of the facts relative to the longevity in association with perfect specimens for study in 99 cases* out of the original 200 cases.

The following facts were recorded upon the 99 cases of the present investigation:

1. Cases with no glandular involvement have a much greater average length of postoperative life.

2. No cases with glandular involvement lived over 8 years.

3. Six per cent of cases without glandular involvement lived over 10 years. Two per cent without glandular involvement lived 11 years or over. One per cent without glandular involvement lived 13 years after operation.

4. No case between 29 and 60 years of age lived over 10 years. Nine per cent of cases between 60 and 72 years of age lived over 10 years. Four and

*Many of the specimens of the original series of 200 had been so mutilated during other studies that they were not in the best condition for this investigation.

seven tenths per cent of cases between 60 and 72 years of age lived over 12 years.

5. The average age of cases with complete glandular involvement is 5 years younger than those cases without glandular involvement.

6. The average age of the youngest cases is the same regardless of glandular involvement.

7. The eldest case with glandular involvement is 5 years younger than the eldest without glandular involvement.

8. The average length of postoperative life is greater between 29 and 40 years of age and 60 and 72 years of age.

In spite of the fact that the length of postoperative life was in inverse relation to the degree of glandular involvement, there was a sufficient number of exceptions to demand further investigation. One case lived over five

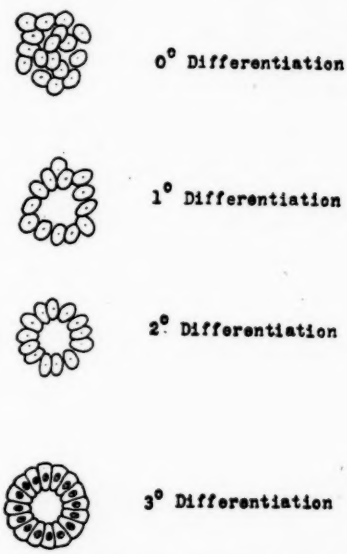

Fig. 4.—Degrees of cellular differentiation based on the stages of differentiation of specific tissues during embryologic development. In the stage without differentiation the cells are merely arranged in masses. In the first degree of differentiation the cells assume the general alignment of the adult tissue but without arrangement of the axes of the cells as in the adult tissue cells. In the second degree of differentiation the axes are parallel or form radii of a sphere or circles. In the third degree the cells assume the adult morphology of the units of the specific tissue.

years and one lived over nine years in spite of the fact that all of the lymphatic glands were involved. The same was true of cases with partial glandular involvement. The question arising from these facts is: What are the factors controlling longevity in these exceptional cases? Doubtless there are many contributing factors to longevity but there are at least two which can be studied with a fair degree of accuracy which may be sufficient to advance our knowledge of the body's defensive mechanism against the destructive character and activities of neoplasmata. In this report, cellular differentiation, and lymphocytic infiltration are considered. There is a general unwritten law in biology which indicates that the power of cellular reproduction is in inverse relation to the degree of cellular differentiation unless the differentiation be for the specific purpose of reproduction. By the term differentiation in biology we

mean structural change for specific function. For convenience of study the degrees of differentiation have been classified according to the visible changes which can easily be recognized in the normal evolution of specific tissues. The differentiation has been divided into three degrees (Fig. 4). The cells of carcinoma sometimes attempt differentiation and it has been thought that this attempt might be seen in the cases which lived unusually long after gastric resection. The specimens were, therefore, studied from this standpoint.

The next factor, as a possible part of the defensive mechanism, is lymphocytic infiltration. The degrees of lymphocytic infiltration are arbitrarily fixed as follows: The first degree, no immediate contact of the lymphocytes with the cancer cells, the lymphocytes being discovered only after careful search of the microscopic fields; second degree, occasional contact of the lymphocytes with the cancer cells; third degree, extensive contact of the lymphocytes with the cancer cells. While this is an arbitrary division of degrees of lymphocytic reaction, the extremes only may be considered to be of possible value since the personal equation, as to the second degree, would be variable in the hands of different observers.

The findings, in so far as the degree of differentiation and lymphocytic infiltration are concerned, were charted with the age, sex, and years of postoperative life.

The three groups were studied separately (see charts) with the following results:

PERCENTAGES ALIVE IN GROUPS (99 CASES)

	GROUP I	GROUP II	GROUP III
Percentage which lived over 1 year	78.7	50	50
" " " " 2 years	60	21.4	30
" " " " 4 "	33	8.9	20
" " " " 6 "	21	5.3	10
" " " " 8 "	12	3.5	10
" " " " 10 "	6	0	0
" " " " 11 "	2	0	0
" " " " 13 "	1	0	0

It may be seen that 78.7 per cent of gastric carcinomata without glandular involvement lived over 1 year; 60 per cent of gastric carcinomata without glandular involvement lived over 2 years; 33 per cent of gastric carcinomata without glandular involvement lived over 4 years; 50 per cent of gastric carcinomata with glandular involvement lived over 1 year; 21-30 per cent of gastric carcinomata with glandular involvement lived over two years; from

	29-40	40-50	50-60	60-72
Percentage alive over 1 year in decades	46	67	67	76
" " " 2 years " "	38	32	32	42
" " " 4 " " "	23	17	17.5	19
" " " 6 " " "	15	14	8	9
" " " 8 " " "	7	3.5	8	9
" " " 10 " " "	0	0	0	9
" " " 12 " " "	0	0	0	4.7
" " " 13 " " "	0	0	0	0

8.9 to 20 per cent of gastric carcinomata with glandular involvement lived over 4 years, or 80-91 per cent died before the end of the fourth year after resection; from 3.5 to 10 per cent of gastric carcinomata with glandular involvement lived over 8 years; and 6 per cent of gastric carcinomata without glandular involvement lived over 10 years.

From these figures it may be seen that the younger the host the shorter the life expectancy after resection for gastric carcinoma regardless of glandular involvement.

CELLULAR DIFFERENTIATION

	I	II	III
Average length of postoperative life in the different groups with no differentiation	3.5 years	1.4 years	2.8 years
Average length of postoperative life in the different groups with differentiation	4 "	1.68 "	2.6 "
Percentage of cases with no differentiation	57.5 "	25 "	20 "
Percentage of cases with differentiation	42.5 "	75 "	80 "

Decade	29-40	40-50	50-60	60-72
Percentage with no differentiation	53.8	25	35	28
Percentage with differentiation	46.2	75	65	72

Average length of postoperative life in all cases with no differentiation.....2.8 years
Average length of postoperative life in all cases with some differentiation.....2.55 "

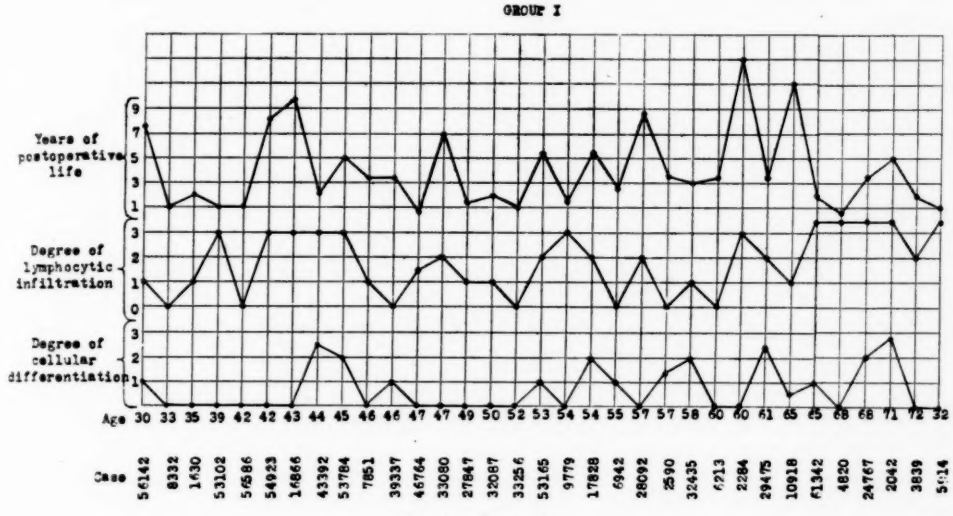

Fig. 5.—Group I.

The facts from these figures may be briefly summarized as follows:

1. Individuals with no glandular involvement and showing differentiation lived 14 per cent longer than those with no glandular involvement and no differentiation.

2. Individuals with partial glandular involvement with differentiation lived 20 per cent longer than those without differentiation.

3. In individuals with complete glandular involvement differentiation was not associated with increased longevity.

4. Differentiation was more frequent in association with glandular involvement (Group II, 42.6 per cent; Group III, 70 per cent).

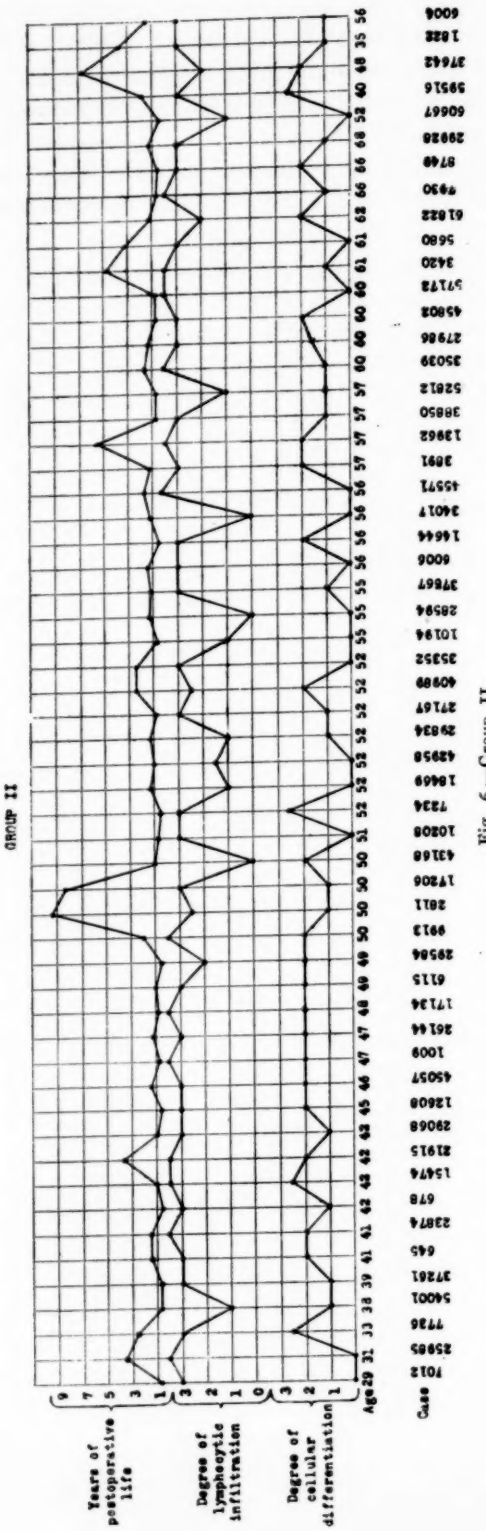

Fig. 6.—Group II.

5. Differentiation was most frequent between the ages of 40 to 50 years and least frequent between 29 to 40 years.

6. In the whole series of cases the average length of life was less (8.9 per cent) with differentiation than without differentiation.

LYMPHOCYTIC INFILTRATION

	I	II	III
Average length of postoperative life in cases with 2 and 2+ lymphocytic infiltration...	5.3 years	4.1 years	4.5 years
Average length of postoperative life in cases with 3 and 3+ lymphocytic infiltration...	4.37 "	1.7 "	2.5 "
Average length of postoperative life in cases no lymphocytic infiltration.....	4.2 "	1.3 "	0.0 "
Average length of postoperative life in all cases with some lymphocytic infiltration			2.7 "
Average length of postoperative life in all cases with no lymphocytic infiltration			2.1 "
Percentage of cases with no lymphocytic infiltration		21 5	0
" " " " 2 or 2+ " "		16 8.9	10
" " " " 3 or 3+ " "		36.3 73.2	80
" " " " 1 or 1+ " "		24.2 12.5	10

Decade	29-40	40-50	50-60	60-72
Percentage of cases with on lymphocytic infiltration	7	3.5	17.5	4.7
" " " " 1 " "	23	14	20	9
" " " " 2 " "	7	10	11	14
" " " " 3 " "	61	71	47	71

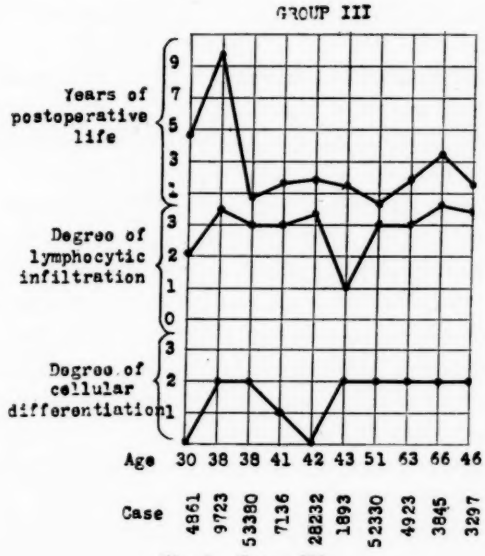

Fig. 7.—Group III.

A study of these figures reveals the following facts in this series:

1. Cases of gastric carcinoma without glandular involvement plus extensive lymphocytic infiltration have the greatest average length of postoperative life (23 per cent longer than when associated with glandular involvement).

2. Regardless of glandular involvement the presence of lymphocytic infiltration is associated with a 23 per cent longer postoperative life.

3. Extensive lymphocytic infiltration is more frequent in association with glandular involvement than it is without glandular involvement.

4. Regardless of glandular involvement extensive lymphocytic infiltration is most frequent between 40 to 50 years and 60 to 72 years.

COMBINATION OF LYMPHOCYTIC INFILTRATION AND DIFFERENTIATION

	I	II	III
Average length of postoperative life with 2 and 2 differentiation and 3 lymphocytic infiltration	4 years	1.5 years	3 years
Average length of postoperative life with no differentiation and lymphocytic infiltration	1.6 "	1.5 "	0 "

From a study of these figures the following conclusions from the series may be made:

The cases of gastric carcinoma with the combination of lymphocytic infiltration, differentiation and no glandular involvement lived 150 per cent longer than the cases with no differentiation and no lymphocytic infiltration.

SUMMARY

1. The average length of postoperative life is 7.5 per cent greater in cases with differentiation than in those without differentiation.

2. There is a much greater percentage of cases in Group I without differentiation.

3. There is a much greater percentage of cases in Group III with differentiation.

4. The greatest frequency of cases with no differentiation occurs in cases between 29 and 40 years of age.

5. The greatest frequency of cases with the greatest amount of differentiation occurs in cases between 40 and 50 years of age.

6. The average length of postoperative life is greatest in cases without glandular involvement plus lymphocytic infiltration.

7. The cases without glandular involvement but with lymphocytic infiltration live 124 per cent longer than those without lymphocytic infiltration.

8. Cases with glandular involvement lived 146 per cent longer when there was lymphocytic infiltration than did those with no infiltration.

9. The highest percentage of cases, without lymphocytic infiltration, were those with no glandular involvement.

10. The highest percentage of cases, with lymphocytic infiltration, were those with glandular involvement.

11. The highest percentage of cases with marked lymphocytic infiltration occurred between 60 and 72 years of age and 40 and 50 years of age.

12. The highest percentage of cases with no lymphocytic infiltration occurred between 50 and 60 years of age.

13. The average length of postoperative life in cases with differentiation and lymphocytic infiltration combined is 82 per cent greater than that without differentiation and lymphocytic infiltration combined.

THE ALKALI RESERVE OF THE BLOOD PLASMA DURING PROTEIN SHOCK*

By A. A. Eggstein, M.D., New York, N. Y.

THE shock produced by the intravenous injection of foreign proteins recently advocated in the nonspecific treatment of chronic infectious processes is frequently associated with symptoms suggestive of an acidosis. In order to determine a decrease in the blood alkalies of animals shocked in this manner a series of investigations have been made of which this communication constitutes a report.

Observations upon the alkali reserve of the blood, during surgical shock, have been made by L. J. Henderson,[1] Yandel Henderson,[2] Crile,[3] Caldwell and Cleveland,[4] Guthrie,[5] McEllroy,[6] Cannon,[7] and others. As surgical and toxemic shock are in all essential features, identical, it is well to review briefly the observations of these investigators. Henderson[2] and coworkers have frequently reported acidosis in surgical shock of animals, produced by experimental means, and his acapnia theory of acidosis based upon these experiments is quite well known. Crile[3] has observed acidosis present in various clinical conditions including shock. Guthrie[5] found a decrease in the reserve alkalinity of the blood in certain cases of experimental shock, but much of the decrease observed occurred after pronounced symptoms of shock had become manifested and that good recovery of cerebral function was possible in shock without a change in the hydrogen-ion or the plasm alkalinity which strongly indicated to the author that the cerebral manifestations of shock are not due to changes in the blood reaction. McEllroy[6] found in experimental shock, a gradual decrease in the alkali reserve of the blood. His experiments led him to the conclusion that acidosis was not a causative factor in the experimental shock studied, and he states that in primary experimental acidosis, where there is a marked lowering of the alkali reserve to a degree lower than that observed in shock, no marked change in the condition of the animal was produced, also that the injection of sodium bicarbonate into animals in shock, was without beneficial action, although the reserve alkali was restored. McEllroy found that shock may be induced even while the alkaline reserve is maintained by the injection of sodium bicarbonate. Cannon's[7] studies included forty-seven cases of low blood pressure in soldiers during the recent war. In some cases the low blood pressure was due to shock alone, while in others it was complicated by hemorrhage or gas-bacillus infection. The relationship of acidosis, blood pressure, respiratory changes and the blood sugar received particular attention in these cases. Cannon also observed the effect of anesthesia and operation upon an existent acidosis and lower blood pressure and the influence of alkaline treatment in cases of ex-

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treme acidosis. In forty per cent of Cannon's cases the mean blood pressure was below 60 millimeters of mercury, and all showed a condition of acidosis. A close parallelism in the drop of the carbon dioxide capacity of the blood, with a drop in the mean blood pressure was found. In ten cases of uncomplicated shock the average carbon dioxide capacity was 41 volume per cent. Upon the basis of some experiments carried out upon soldiers in shock, Cannon highly recommends the preliminary administration of alkalies in order to combat the extreme acidosis that follows operations on men in surgical shock.

TECHNIC

In these experiments, dogs were used chiefly. These dogs were kept in the laboratory kennel upon a well balanced diet, for a period not less than a week. The carbon dioxide capacity of the plasma was determined according to the technic of Van Slyke.[8] The animals were bled from the jugular vein, by means of a syringe and the blood immediately mixed with neutral potassium oxalate, and covered with liquid paraffin. The blood was tested previous to the production of the shock and at regular intervals following. Secondary proteoses and typhoid bacteria were the toxic substances used to produce shock and were given intravenously.

EXPERIMENTS

TABLE I

Dog 1, weight 5 kg.

EXPERIMENT TIME	INJECTION	C.C. OF CO_2 CHEMICALLY BOUND BY 100 C.C. PLASMA
9:00 A.M.		50.4
9:10 A.M.	1.0 gm. proteoses	
2:00 P.M.		25.8
3:00 P.M.		30.0
6:00 P.M.		38.1
9:00 A.M.		50.0

Table I shows a marked acidosis in a dog, following proteose shock. Similar experiments were repeated upon a series of eight dogs with slight variations in results. Therefore it is considered sufficient to present in tabular form the average figures obtained from the eight experiments.

TABLE II

Average weight of eight dogs, 5.4 kg.
Average dose of proteoses, 0.2 gms. per Kg.

TIME INTERVALS	INJECTION	C.C. OF CO_2 CHEMICALLY BOUND BY 100 C.C. PLASMA
Before injection		54.8
10 minutes later	Proteoses	
2 hours later		32.4
8 hours later		40.4
24 hours later		45.0
48 hours later		53.2

Both Tables I and II show a marked decrease in the carbon dioxide capacity of the plasma of dogs following shock produced by the intravenous injection of toxic proteoses. Seven of the nine dogs used in these two ex-

periments, showed a drop in the carbon dioxide capacity of the plasma. In one dog included in Table II, receiving the same amount of proteoses proportionately as the others, there was practically no change in the alkali reserve of the blood. However, this animal presented definite evidence of acute shock. In spite of this unexplained variation in the one animal, the table shows a rapid and continuous decline in the alkaline reserve of the blood. The animals usually recover from this condition of acidosis in less than twenty-four hours. The symptoms presented by the animals were vomiting, diarrhea, restlessness, dilated pupils, prostration, low blood pressure. One of the animals died during the night following the shock. When a decrease in the alkali reserve was below 20 per cent the animals usually showed severe clinical symptoms and the animal which died showed a carbon dioxide capacity decrease of 56.7 to 18.3. Blood pressure tracings were made on several dogs, and in these animals there was a drop in the pressure following the injection of the proteoses which was closely associated with a drop in the alkali reserve of the plasma. This is similar to the findings of Cannon in surgical shock. The failure of one of the animals in the series to show a decrease in the alkali reserve following the proteoses injection remains unexplained. However, Guthrie[5] reports an irregular production of acidosis in dogs during surgical shock.

The results obtained upon proteose shock in dogs suggested the use of other toxic proteins upon the alkali reserve of the blood, and in the following experiments, typhoid vaccine was employed intravenously to produce a severe shock.

Below are tabulated the average figures of seven animals shocked by the intravenous injection of typhoid bacteria.

TABLE III

Average weight of seven dogs, 6.5 Kg.
Average dose per Kg., 40,000,000 dead typhoid bacteria.

TIME INTERVALS	INJECTION	C.C. OF CO_2 CHEMICALLY BOUND BY 100 C.C. PLASMA
Before injection		55.3
10 minutes later	Typhoid bacteria	
2 hours later		29.3
8 hours later		40.2
24 hours later		53.8
48 hours later		48.6

There is a marked drop in the carbon dioxide capacity of the plasma of dogs receiving an intravenous injection of typhoid bacteria as shown in Table III. In one dog of this series, the carbon dioxide capacity fell from 60.5 volume per cent to 19.5 volume per cent. This animal died the night following the injection of the bacteria. Another one of the dogs, in which the carbon dioxide capacity had dropped to 26.02 volume per cent, died. There was marked prostration in all of these animals. The symptoms were more severe following the typhoid than the proteose injections. The typhoid-shocked animals showed more marked diarrhea, the stools containing a large amount of blood. There was a greater diminution of the alkali reserve of the plasma in the animals receiving the typhoid vaccine, than those shocked by proteoses.

HUMAN CASES

Having demonstrated changes in the alkali reserve of the plasma of dogs following protein shock, it was considered advisable to study the reaction of the plasma following the therapeutic administration of toxic proteins in patients.

A negro woman, aged forty, suffering from chronic arthritis, with carbon dioxide capacity 55.8 volume per cent before an intravenous injection of twenty million dead typhoid bacilli. The injection was followed by chill, headache and general malaise, and a rise in temperature of two degrees. One hour after the injection, the plasma carbon dioxide capacity was 45.9. Similar experiments upon a group of eight patients were repeated with similar results. All of the patients showed a slight decrease in the carbon dioxide capacity of the plasma following this form of shock.

THE EFFECT OF THE ADMINISTRATION OF SODIUM BICARBONATE

From the observations of the above experiments it was thought desirable to determine the effect of the administration of sodium bicarbonate upon these reactions when given intravenously to the animals before and after the production of a shock.

TABLE IV

Average weight of four dogs, 6.2 Kg.
Average dose of proteoses, 0.2 gms. per Kg.

TIME INTERVALS	INJECTIONS	C.C. OF CO_2 CHEMICALLY BOUND BY 100 C.C. PLASMA
Before injection		60.3
10 minutes later	2 gms. sodium bicarbonate	
30 minutes later		75.8
45 minutes later	Proteoses	
1½ hours later		40.2
8 hours later		50.4
24 hours later		62.6

TABLE V

Average weight of four dogs, 7 Kg.
Average number of typhoid bacilli per Kg., 400,000,000.

TIME INTERVALS	INJECTION	C.C. OF CO_2 CHEMICALLY BOUND BY 100 C.C. PLASMA
Before injection	2 grs. sodium bicarbonate	69.1
15 minutes later	typhoid bacteria	
35 minutes later		76.4
1 hour later		
1½ hours later		35.6
10 hours later		59.7
22 hours later		63.4

Eight dogs were taken, and the carbon dioxide capacity of the blood determined. The dogs were given sodium bicarbonate and the rise in the alkali reserve of the blood noted. In four of these dogs, proteoses were given and in the other four, typhoid vaccine. The blood plasma carbon dioxide capacity of these dogs showed a drop to a point far below the capacity before the ad-

ministration of the alkali. Clinically, the eight animals showed a distinct protein shock, with little or no change in the clinical symptoms, but the carbon dioxide capacity did not reach as low a level as in the untreated. In the tables are shown the composite results of these experiments.

The next experiments were made to determine the effect of the administration of alkali after the production of shock in dogs. It will suffice to give the following representative experiment which shows the results obtained in a group of five dogs.

TABLE VI

Weight of dog, 6 Kg.
Number of typhoid bacteria, 400,000 per Kg.

TIME INTERVALS	INJECTION	C.C. OF CO_2 CHEMICALLY BOUND BY 100 C.C. PLASMA
Before injection		56.1
18 minutes later	Typhoid vaccine	
40 minutes later		30.3
1 hour later	2 grs. sodium bicarbonate	
1 hr. 20 min. later		40.5
9 hours later		45.6
20 hours later		52.4

In Table VI is represented a very rapid decrease in the alkali reserve of the plasma of a dog after vaccine shock with a subsequent rise after the administration of sodium bicarbonate. All the animals showed severe clinical symptoms after the injections of the typhoid vaccine. Several of the dogs showed an apparent improvement of certain clinical symptoms immediately following the alkali treatment. Dyspnea was less, blood pressure higher, vomiting and diarrhea not so severe, as in the animals not receiving alkali. However, all the toxic symptoms were not relieved, and several of the dogs remained prostrated for several hours.

DISCUSSION

From these experiments it is evident that in protein shock, there is a decrease in the alkali reserve of the blood plasma. These findings correspond with the observations of Crile, Cannon, Wright and others, upon the alkali reserve of the blood following surgical shock and in infectious diseases. From these experiments, the decrease in the alkali reserve in infectious disease, is probably due to the liberation of toxic proteins into the circulation. The sources of the toxic proteins, being a bacteriemia or the incomplete autolysis of the necrotic cells of tissues and inflammatory exudates. These experiments indicate that proper attention should be paid to the alkali reserve of the blood in the treatment of toxemic conditions and infectious diseases, particularly typhoid fever and pneumonia, as in these conditions large amounts of toxic proteins gain entrance into the circulation.

As a result of the improvement of certain distressing symptoms in dogs, after the administrations of alkalies, it would seem advisable to administer alkali in the treatment of infectious diseases and other toxemias for the relief of respiratory and circulatory symptoms so frequently found in these conditions.

CONCLUSION

1. The alkali reserve of the blood plasma is greatly decreased in shock, following the intravenous injection of toxic proteoses and typhoid vaccines in dogs and in human cases.

2. There was found a definite relationship between the decrease in the alkaline reserve of the plasma and the lowered blood pressure in toxemic shock.

3. When the alkali reserve of the blood falls below thirty volume per cent, following protein shock, the animal's life is in danger.

4. The administration of sodium bicarbonate preliminary to the injection of a toxic protein retards the fall of the blood alkalies to this critical point.

5. When the alkali reserve has been lowered in protein shock, it may be restored by the intravenous administration of sodium bicarbonate, which apparently relieves distressing symptoms.

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STUDIES ON THE RESISTANCE OF THE RED BLOOD CELLS*

II. The Resistance of the Red Blood Cells in Disease to the Hemolytic Action of Sapotoxin

By Chas. H. Neilson, M.D., and Homer Wheelon, M.D., St. Louis, Mo.

IN a previous communication a rapid method was given for the determination of the resistance of the red blood cells to sapotoxin solutions.[1] In brief the method consisted of exposing a given amount of whole blood, 20 c. mm., for a period of 5 minutes to various strengths of sapotoxin solution at a constant temperature—25° C. With this method the red cells of normal whole blood of 185 determinations showed an average minimal degree of hemolysis in a 1:13,937 sapotoxin solution. It was also shown that the resistance of washed cells was markedly less than that of those suspended in normal blood fluids. Such results led to the conclusion that the blood fluids acted in such a manner as to protect or raise the resistance of the red blood cells to the hemolytic action of sapotoxin.

The present paper deals with the relation of red cell resistance in various of the common diseases to the hemolytic action of sapotoxin. Brief mention is made concerning the relation of cholesterol to the hemolytic action of sapotoxin inasmuch as the cholesterol content of the blood in certain of the diseases was found to vary in proportion to variations in red blood cell resistance.

Experimental Results

I. Pregnancy and the Resistance of the Red Blood Cells

While studying the resistance of the red blood cells of patients suffering from pulmonary tuberculosis we were impressed by the great degree of resistance offered by two women both of whom were pregnant. No. 77 (8 months pregnant) demonstrated a resistance of 1:10,500, the other, No. 78 (6 months pregnant) 1:10,000. The average resistance of 34 tuberculous patients was 1:12,375, an increase above the normal of 1,562 points, hence, tuberculosis and pregnancy each tend to accentuate the resistance of the red blood corpuscles to sapotoxin solutions.

The laking reaction of red cells in whole blood to sapotoxin solutions was determined upon 41 cases of pregnancy. In all 139 determinations were made during the latter days of pregnancy, labor, and the 4th, 8th, and 12th days postpartum. Thirty-five tests made within two weeks of delivery gave an average resistance of 1:11,271 solution. The time for complete hemolysis to occur in a 1:13,000 solution was 16.8 minutes. The average hemoglobin was 89 per cent as determined by the Tallquist hemoglobinometer. (See Table I and Fig. 1.)

*From the Department of Medicine of the St. Louis University School of Medicine, St. Louis, Mo.

Ten cases were studied during the period of labor. The average strength of sapotoxin solution required to hemolyze the red cells in five minutes was a 1:10,050 solution. Complete laking occurred in the 1:13,000 solution in 19.2 minutes; average hemoglobin 90 per cent. Hence, during parturition there is an increased resistance to sapotoxin solutions of 1,221 points above that of the last two weeks of the prepartum period and 3,887 points increase over the

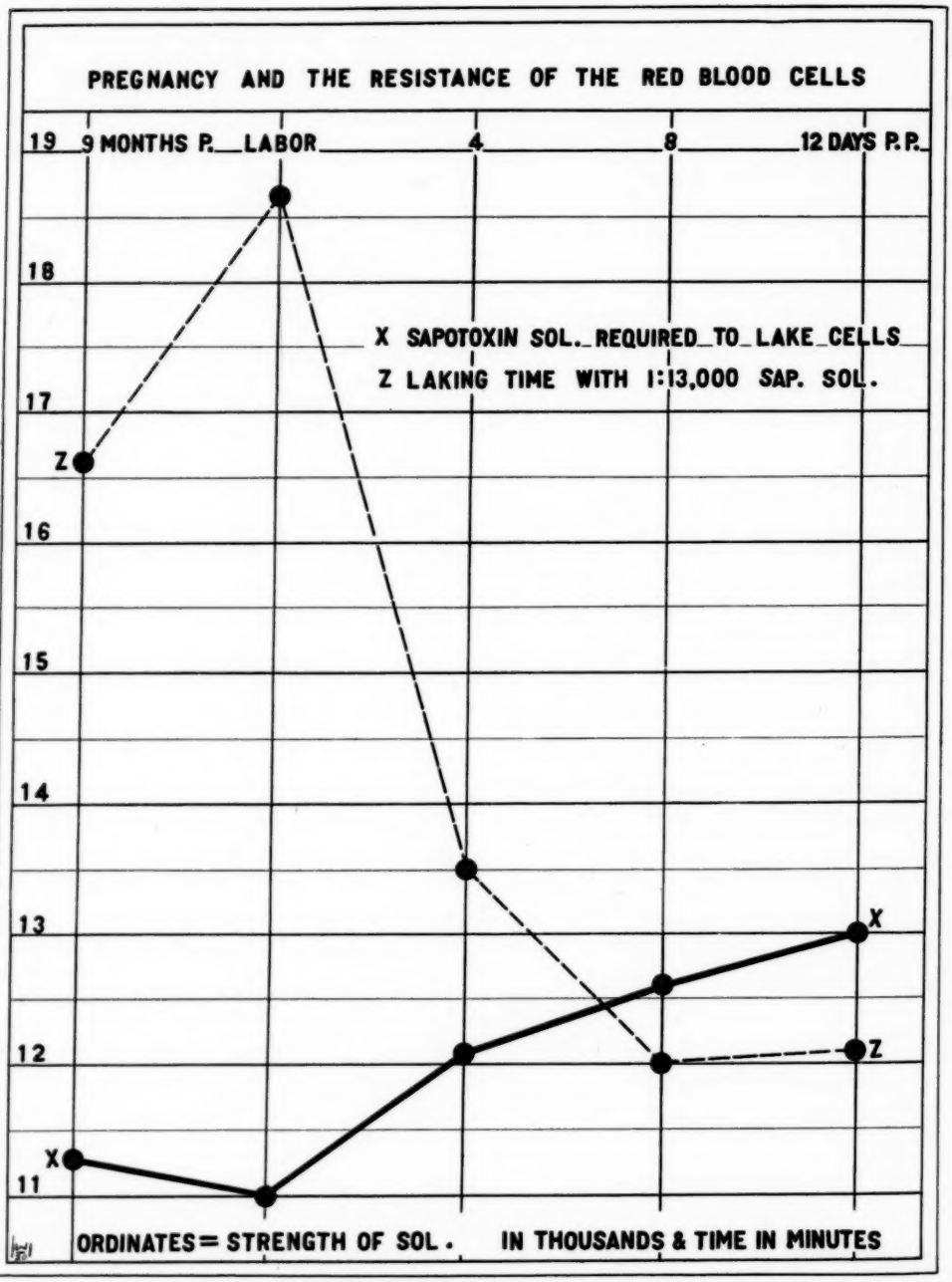

Fig. 1.—Curves indicating the resistance of the red cells during pregnancy and the first 12 days postpartum.

average normal. The length of time required for complete laking of the red cells in a 1:13,000 solution was also increased by 2.4 minutes.

Thirty-three determinations made on the fourth day postpartum gave an average resistance strength of 1:12,060 solution. The average laking time in a 1:13,000 solution was 14.1 minutes; hemoglobin averaged 88 per cent. Hence the blood cells were less resistant by 1.010 points than during the period of delivery; 789 points less than the prepartum period and 1.877 points higher than the average normal. The time for complete laking in the 1:13,000 solution was decreased by 5 minutes over the time required at the time of delivery and 2.6 minutes less than the prepartum determinations.

TABLE I

PREGNANCY AND RED CELL RESISTANCE TO SAPOTOXIN SOLUTIONS

	NO. CASES	RESISTANCE	1:13,000	HB.
Prepartum	35	1:11,271	16.8	89
Labor	10	1:11,050	19.2	90
Postpartum				
4th Day	33	1:12,060	14.2	88
8th Day	31	1:12,581	12.2	90
12th Day	30	1:13,000	11.8	87
Average of all	139	1:11,933	14.9	89
Normal	185	1:13,937	10.7	91

Resistance, Strength solution showing minimal hemolysis. 1:13,000.
Time in minutes for complete hemolysis to occur. Hb., Hemoglobin.

On the eighth day postpartum 31 tests gave an average resistance strength of 1:12,581 solution; a decrease of 1,310 points below the prepartum period and 1,356 points above the average normal. The average time for complete laking in the 1:13,000 solution was 12.2 minutes, or 4.6 minutes less than the prepartum period. Average hemoglobin, 90 per cent.

Thirty determinations made on the twelfth day postpartum gave an average laking strength of 1:13,000 solution, a decrease of 1,729 points below the prepartum period and 937 points above the average normal. The average laking time for a 1:13,000 solution was 11.8 minutes; a decrease of 5 minutes below the prepartum period.

Early pregnancy appears to increase the resistance of the red blood cells to sapotoxin solution. The average resistance of 18 cases of pregnancy between the third and seventh month was a 1:12,139 solution: an increase of 1,798 above the average normal, or 868 points lower than the average resistance for the period just prior to parturition. The average hemoglobin was 86.4 per cent and the average complete hemolysis in a 1:13,000 solution was 13.1 minutes.

Inasmuch as obstetrical cases usually leave the hospital 14 days after delivery it was not possible to carry the observations over a longer period of time. At the end of twelve days the resistance of the red blood cells is materially reduced below that of the prepartum period; however, it still shows a slight increased resistance above that of the average normal.

The work of Hymanson and Kahn[2] shows that the mean total lipoid content of the maternal blood is 0.475, cholesterol 0.219 per cent. The blood from the newborn infant gives practically identical readings. According to

Bloor and Knudson[3] there is a constant relation between the free cholesterol and cholesterol esters of human blood. In whole blood the average percentage of cholesterol in combination as esters is 33.5 per cent, and in the plasma 58 per cent of the total cholesterol content. In pregnancy the values for cholesterol esters are high. Hypercholesterolemia is common in pregnancy although not invariable. The relation of gallstones to pregnancy is of common observation.

II. MALARIA AND THE RESISTANCE OF THE RED BLOOD CELLS

Inasmuch as malaria is accompanied by more or less severe destruction of the red blood cells by the malarial plasmodium, it was considered of interest to test the resistance of the erythrocytes before and after the administration of quinine. The relation of red cell destruction and quinine in the production of "black-water fever" of necessity brings up the question of an active hemolysis in such cases.

Ninety-eight blood resistance determinations were made upon 38 malarial patients. Twenty-three positive malarial cases upon whom tests were made prior to the administration of quinine gave an average minimal hemolysis reaction in a 1:14,380 sapotoxin solution. Apparently, therefore, the presence of the infection does not greatly alter the resistance of the erythrocytes as there is but 443 points difference in such cases and the average normal.

The first day following the taking of massive doses of quinine (15 to 60 grs.), 35 blood determinations gave an average minimal resistance of 1:15,022. On the second or third day following quinine medication the resistance of the erythrocytes fell to an average of 1:15,417, or a loss of 1,037 points below the average prior to the taking of the quinine and 1,470 points below the average normal. Following the initial reduction, the resistance of the red cells gradually returned and demonstrated a minimal hemolysis in a solution of 1:14,750 at the end of a week's medication.

Following the withdrawal of quinine, the resistance of the red cells gave an average reading of 1:14,409, a resistance equivalent to that demonstrated during the time of malarial invasion and prior to the taking of quinine. "Black-water fever" did not develop in any of our cases, hence, no information was obtained relative to that point.

As a check upon the above experiments, the effects of quinine on seven nonmalarial persons was determined. These persons received the same dosage of quinine and were tested the same as the malarial patients. The average resistance of these controls prior to the taking of quinine was 1:14,142. Within 24 hours after taking quinine the resistance of the red cells was increased to a 1:13,893 solution; a figure within the range of normal determinations. At no time during the entire week of medication did the average resistance move out of the range of normal variations. The withdrawal of quinine had no apparent effect upon the red cells as the average resistance at such a time was 1:13,937 or the same as the average normal. (See Table II and Fig. 2.)

Hemoglobin is apparently but little affected by quinine, although our series of patients did demonstrate an average gain of 7.3 per cent in hemo-

globin following heroic quinine medication. Such a gain may be accounted for because of the cessation of red cell destruction by the malarial plasmodium.

Lovelace,[4] in a study of 514 cases of hemoglobinuric fever found that malarial infection stands in a direct causal relation to "black-water fever".

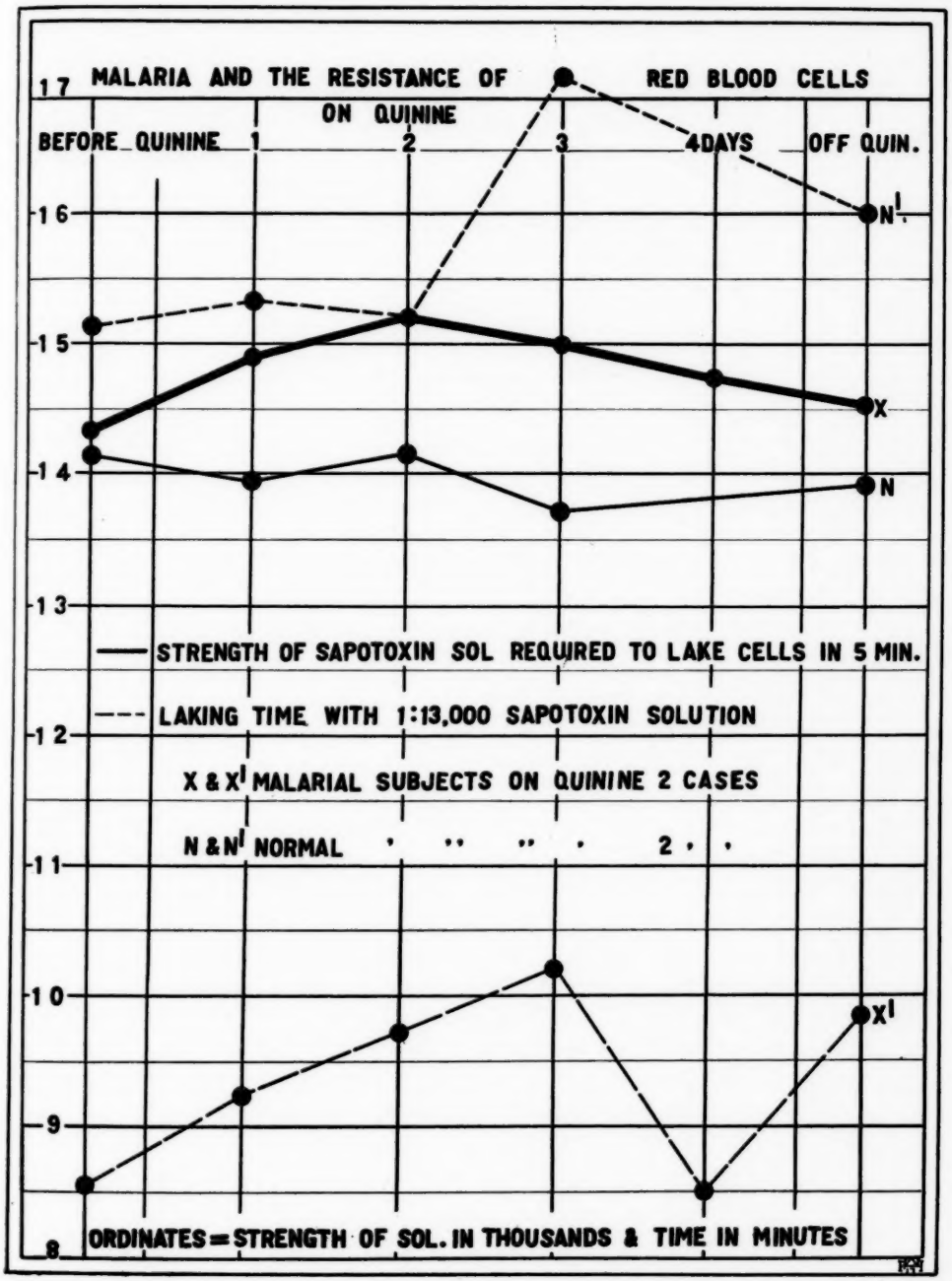

Fig. 2.—Curves showing the effect of quinine on the resistance of normal and malarial blood cells to sapotoxin. The 2 nonmalarial cases received the same treatment as the 2 malarial cases. The legends of the figure explain the curves.

TABLE II

MALARIA AND RED CELL RESISTANCE TO SAPOTOXIN SOLUTIONS

	NO. CASES	RESISTANCE	1:13,000	HB.
Malaria	23	1:14,380	11.6	86
1st Day on Quinine	35	1:15,022	8.5	89
During Week on Quinine	32	1:15,242	8.9	89
Off Quinine	8	1:14,409	9.3	90
Average of all	98	1:14,763	9.5	89
Normals	185	1:13,937	19.7	91
	NONMALARIAL PATIENTS ON QUININE			
Before Quinine	7	1:14,142	15.0	86
1st Day on Quinine	7	1:13,893	15.3	88
During week on Quinine	5	1:13,944	5.7	88
Off Quinine	4	1:13,937	16.00	86
Average of all	23	1:13,979	13.0	87

The administration of quinine either in large or small doses, was, in his cases, an invariable antecedent of the hemoglobinuric condition. Brem,[5] on the other hand, does not believe that quinine acts as an etiologic factor, either predisposing or exciting. However, Brem does believe that previous attacks of malaria, especially of the estivo-autumnal type, do act as predisposing causes of hemoglobinuric fever. He also advanced the theory that the malarial organism probably excited the production of an acute hemolysin, which in turn destroyed the red cells. The views of Cardamatis[6] concerning the etiology of black-water fever agree with those of Lovelace, namely, quinine and malaria. Cardamatis found the mortality in cases of black-water fever treated with quinine 23.6 per cent, while in cases treated without quinine the rate of mortality was but 7.5 per cent.

The relation of paroxysmal hemoglobinuria to syphilis is of interest in that syphilis like malaria is the result of a protozoan parasite. Hemoglobinuria is also known to occur in various protozoon invasions of animals. In such cases the development of an autolysin or hemolysin gives rise to the destruction of the subject's own corpuscles. As will be shown more fully later, the degree of resistance of the red cells to laking agents is materially altered in cases of syphilis not under treatment. This loss of resistance, or increased fragility, is especially marked when the washed cells are studied.

Butler[7] reports that the resistance of the red corpuscles to salt solutions is normal in malarial cases. Sahli[8] in discussing this point says: "The determination of the power of resistance of the red blood cell to hypotonic injury is not sufficient to settle all questions in reference to their resisting power, since Chvostek, 1899, in contrast to Murin, found that in paroxysmal hemoglobinuria the power of resistance of the red blood cells to salt solutions is normal, while they show but slight resistance to mechanical injury (shaking) and to stasis within the body."

III. TUBERCULOSIS AND THE RESISTANCE OF THE RED BLOOD CELLS

Reports relative to the resistance of the red cells in tuberculosis to salt solutions are conflicting. It is more than probable that factors other than

tuberculosis are to be considered in such cases; the degree of anemia, cachexia, malignancy, jaundice, syphilis and pregnancy all affect the resistance of the red cells. Hence, a disturbance of tissues other than those affected by tuberculosis may account for the variations in results that have been reported. Pregnancy, as previously shown, does increase the resistance of the red blood corpuscles to a striking degree. This observation has also been reported by other observers.

The average resistance of erythrocytes from 24 patients suffering from pulmonary tuberculosis was 1:12,375, an increase of 1,562 points above the average normal. The time for complete hemolysis to occur in a 1:13,000 solution was 13.1 minutes or an increase of 2.4 minutes above the average normal. The hemoglobin determinations averaged 81.6 per cent (Table III).

Two patients (Nos. 77 and 78) suffering from pulmonary tuberculosis were pregnant, one 5 months, the other 8 months. No. 77 (8 months) demonstrated a resistance of 1:10,500; an increase of 3,437 points above the normal and 1,875 points above the average for the 24 tuberculous cases. No. 78 (5 months) gave a resistance of 1:10,000, an increase above the average normal of 3,937 points or an increase above the average for the tuberculous patients of 2,375 points. In the light of the results obtained from the study of pregnancy these two cases show not only an increased resistance of the red blood cells to sapotoxin because of the tuberculous condition but also because of pregnancy.

Cases Nos. 523 and 827 were both tuberculous and luetic and were receiving treatment accordingly. The resistance of the red cells in these two cases was 1:14,000 and 1:13,750, respectively. Hence Case No. 523 demonstrated a reading which fell within the range of the average normal, but demonstrated a decrease of 1,625 points below the average resistance of the 24 tubercular cases. Case No. 827 showed a resistance of 1:13,750; an increase of 187 points above the normal average of 1:13,937, or a decrease of 1,375 points below the average for tuberculous patients. The low hemoglobin may in part account for these findings. Hence, lues in some way reduces the resistance of the red blood cells in cases suffering from this disease in conjunction with tuberculosis. (See Section V.)

TABLE III

PULMONARY TUBERCULOSIS AND RED CELL RESISTANCE TO SAPOTOXIN

	NO. CASES	RESISTANCE	1:13,000	HB.
All cases	24	1:12,375	13.1	82
Lues and pregnancy	19	1:12,312	11.4	83
Tuberculosis and lues	2	1:13,875	15.8	68
Tuberculosis and pregnancy	2	1:10,250	23.3	83
Normals	185	1:13,937	10.7	91

IV. TYPHOID FEVER AND RED BLOOD CELL RESISTANCE

Conflicting results have been reported relative to the degree of resistance of the red blood corpuscles in cases of typhoid fever. The usual results seem to be about the same as those for normal individuals. However, it appears that

TABLE IV

TYPHOID FEVER AND RED CELL RESISTANCE TO SAPOTOXIN

	CASES	RESISTANCE	1:13,000	HB.
All Stages of Typhoid fever	19	1:13,513	10.8	85
Normals	185	1:13,937	10.7	91

during the period of convalescence there is a slight increase in the resistance of the red cells, also an increase in the cholesterol content of the blood.

The resistance of the red blood cells to sapotoxin solutions was determined in 19 positive cases of typhoid. The average resistance of the series was 1:13,513, or an increase of 424 points—readings within the normal range of variation. The average time for complete hemolysis to occur in a 1:13,000 solution was 10.8 minutes. The average hemoglobin was 85 per cent. The greatest resistance shown was in Cases 127 and 297 in which the resistance was 1:12,000 in each. Both patients were running high temperatures but otherwise quite comfortable. The greatest loss of resistance occurred in Case 763 who was doing badly and soon died. His resistance was 1:15,750.

Hemolysis determinations at times showed an increased resistance of the cells during the period of convalescence; however, variations at any time were not great. Therefore, typhoid fever appears to alter but little the resistance of the red blood cells to the laking action of sapotoxin solutions. Conditions complicating typhoid fever seem to alter the resistance of the red cells, but no definite conclusions could be derived from our study. (Table IV.)

V. SYPHILIS AND THE RESISTANCE OF THE RED BLOOD CELLS

The resistance of the red blood cells to sapotoxin solutions was determined in 61 cases of syphilis. The average resistance in 34 positive cases prior to medication was 1:14,699, or 762 points lower than the average normal. Fifty-six determinations made upon 23 patients under mercurial treatment alone gave an average red cell resistance of 1:13,659. This represents an increase of 1,040 points over the average untreated patients and 278 points increased resistance over the average normal. The average resistance of 24 determinations upon positive cases of syphilis under a treatment of mercury and iodides was 1:14,750. The addition of iodides to the treatment, therefore

TABLE V

SYPHILIS AND RED CELL RESISTANCE TO SAPOTOXIN SOLUTIONS

	CASES	RESISTANCE	1:13,000	HB.
Positive lues no treatment	34	1:14,699	10.2	82
On mercurials	56	1:13,659	14.0	88
On mercury and Iodides	13	1:14,750	10.7	84
On Iodides	11	1:13,977	9.4	86
Average	114	1:14,271	11.7	86
CONTROLS ON POTASSIUM IODIDE 15 M. SAT. SOL. THREE TIMES A DAY				
Normal	4	1:14,625	11.2	90
On Iodides	4	1:15,375	14.6	90
Normal	185	1:13,937	10.7	91

caused a decrease of 1,091 points in the resistance of the blood cells below the average during mercurial treatment alone, or a reduction of 813 points below the normal average. (Table V and Fig. 3.)

The effect of potassium iodide on the red cell resistance was determined on 4 normal non-luetic persons. These four cases received 15 mm. three times a day for

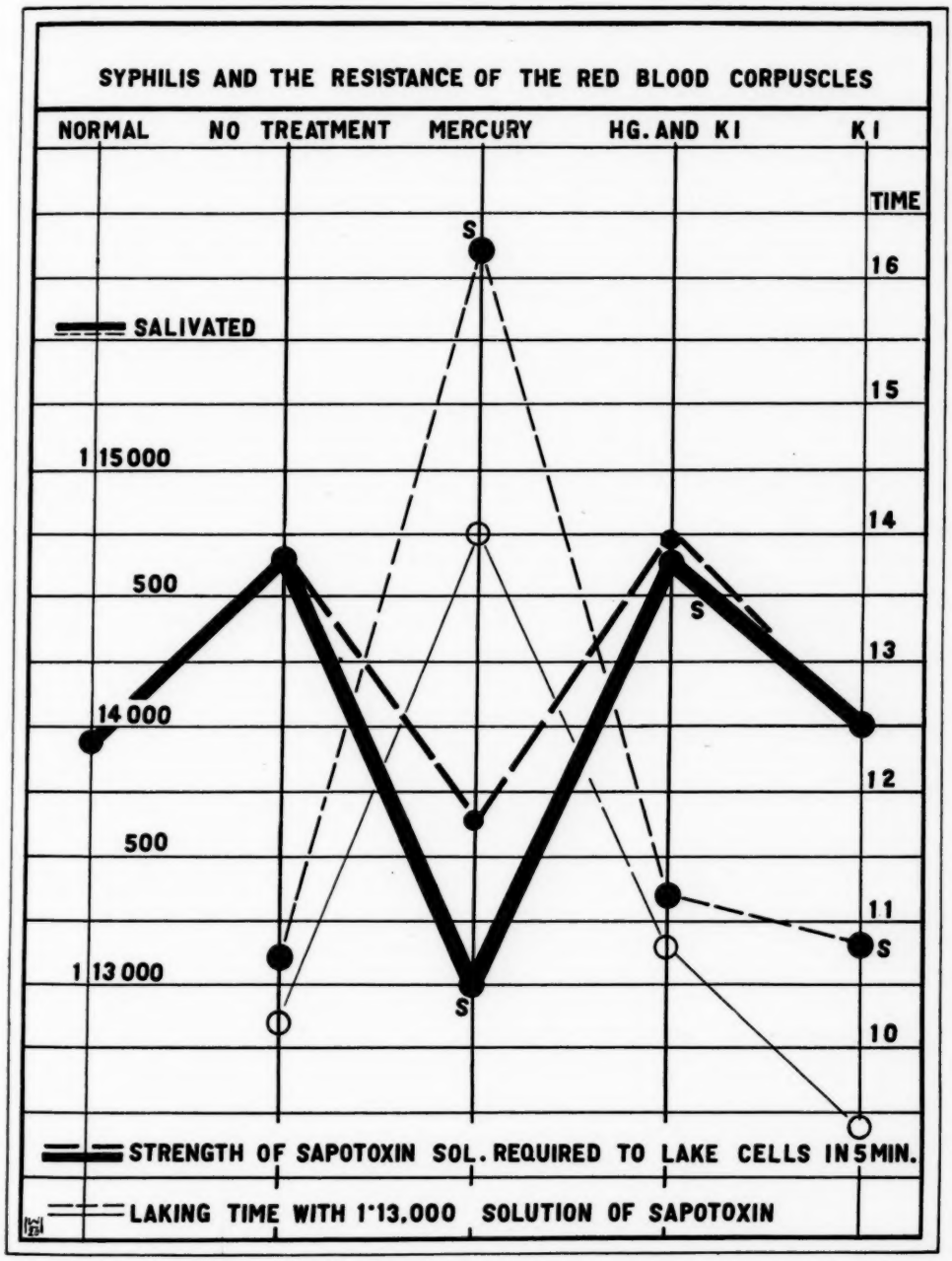

Fig. 3.—Curves showing the effect of mercury and the iodides on the resistance of the red cells of syphilitics. The results of salivation in two cases are shown by curves so indicated. The remaining curves represent the average reactions of all cases studied under the various treatments.

two days. The determinations were made from two to three hours after the last dose. The average normal resistance of these men was 1:14,625—698 points below the average normal. Determinations made after the medication gave a decrease of 750 points in the resistance determinations. The average hemoglobin determination was 90 per cent. Hence the experimental use of iodides resulted the same as in luetic cases following the administration of mercurials.

VI. LEAD POISONING

Seven cases of lead poisoning gave an average resistance of 1:14,321. Five of these cases were receiving belladonna and magnesium sulphate treatment; these gave an average resistance of 1:14,321 solution. The 2 cases untreated averaged a resistance of 1:13,125, or 812 points greater than the normal average.

VII. JAUNDICE AND THE RESISTANCE OF THE RED BLOOD CELLS

Chauffard[9] working with Ribierre's method for the determination of the resistance of the red cells to hypotonic salt solutions, found that obstructive jaundice could be differentiated from other types, designated by him as "hemolytic", by the fact that in obstructive jaundice the resistance of the red cells was raised, while in hemolytic jaundice it was lowered. He also reports that the red cells show a lessened resistance in pernicious anemia with jaundice, and in the so-called "Hereditary jaundice of Minkowski". According to Sommer[10] the red cell resistance is definitely lowered in diseases accompanying elevated blood pressure, and especially in hemolytic icterus.

Hypercholesteremia is invariably present in cases of cholelithiasis. Gorham and Myers[13] found the cholesterol content to vary in such cases. Cases of congenital jaundice show a marked lowering of the cholesterol content of the blood. De Langen[11] reports that gallstone complaints are seldom seen among the Javanese and that the cholesterol content of the blood is only one half the quantity found in European blood. The bile also has a low cholesterol content.

Unquestionably the liver and spleen in mammals in some way aid in the destruction of red cells. However, the removal of the spleen does not prevent the formation of bile pigments. The bile pigments are derived from hemoglobin; hence, it appears that alterations in the functioning of the liver give rise to alterations in the resistance of the erythrocytes to hemolytic agents. Certainly one of the functions of the liver is to keep the various constituents of the altered red cells innoxious for blood cell stroma and hematoporphyrins are, in themselves, toxic and hemolytic in nature. If the bile ducts are ligated, bile pigment and other salts rapidly accumulate in the blood of mammals and birds, on the other hand, ligation of the portal vein and hepatic artery does not result in an accumulation, and bile pigments do not occur in the urine. Any means which leads to the setting free of hemoglobin in the blood causes an increase in the excretion of bile pigments. Injection of certain chemicals and inhalations of arseniureted hydrogen cause marked hemolysis, which may be carried to the point of hemoglobinuria and an in-

crease in the secretion of bile. Injections of hemoglobin produce the same results. Hence, red cell destruction means increased bile pigment formation, and if for any reason the flow of bile from the ducts is hindered, the liver is no longer able to rid itself of its own action and jaundice results.

Jaundice, however, appears to result because of two widely different causes; (a) by occlusion of the bile passages, and (b) by specific hemolytic processes. Perhaps the underlying causes are the same in both cases. Hemolytic jaundice may be the result of accumulation of materials in the blood in excess of the power of the liver to properly care for them in the manufacturing of bile. The cholesterol metabolism is at fault, and this allows the unchecked action of hemolytic agents upon the red blood cells. The cholesterol of the bile may be in part derived from the food and in part from the destroyed blood cells. However, the bile is able to dissolve more cholesterol than is usually found in it. This power of solution of bile depends on the presence of bile salts and specifically upon the cholic acid radicle of the salts. The cholic acid portion of such salts is closely associated with cholesterol, and because of this the cholesterol is maintained in solution. Bile salts are in themselves hemolytic and act similar to the saponins which form easily dissociable compounds with cholesterol. Therefore, it is probable that cholesterol unites in a similar way with the hemolytic group of the bile salts thereby forming a soluble compound in the bile. If such is true, the accumulation of bile salts and a reduction of cholesterol may be, in part, at least, the cause of certain types of jaundice.

The average resistance of the red blood cells as determined by 31 tests upon 27 jaundiced patients was a 1:12,685 sapotoxin solution. This gives an average increased resistance of 1,252 points above the average normal (Table VI). The average hemoglobin was 79 per cent. It was found practically impossible to determine the time for complete hemolysis in a 1:13,000 solution. Usually the blood coagulated and hemolysis was suspended; this was especially true for blood drawn from patients with gallstones. The average for 19 such determinations was 55 minutes. This, however, cannot be considered as at all accurate.

Fifteen positive cases of gallstones gave an average resistance of 1:10,033, or an increase above the average normal of 3,904 points.

Five cases of hemolytic jaundice gave an average resistance of 1:18,600 or a decreased resistance of 4,663 points below the average normal and 8,567 points below the average for obstructive jaundice cases.

Eleven miscellaneous cases of jaundice not definitely diagnosed gave an average resistance of 1:13,182. The variations in resistance were great in this series of cases.

Four cases of cirrhosis of the liver gave an average resistance of 1:14,500, —563 points below the normal average.

The red cells from 4 patients suffering from obstructive jaundice when washed gave an average resistance of 1:36,750 sapotoxin solution. The average resistance of 12 determinations made upon blood from normal individuals was 1:37,375. Hence, there is but little difference in the resistance of washed

cells whether taken from normal or jaundiced cases. However, red cells exposed to their own blood fluids show a great degree of resistance in cases of obstructive jaundice (1:10,033). The relation of the blood fluids to the resistance of the red cells is shown graphically in Table VII. From the table it will be noted that the washed red cells of Case X when placed in the serum of Case Y offered a greater resistance than when placed in their own serum. On the other hand, the washed cells of Case Y when suspended in the serum of Case X which contained less cholesterol offered a less resistance to sapotoxin than when placed in their own serum. Hence, the contents of the blood fluid acts as an antihemolytic agent. In the case of obstructive jaundice it appears that the increase in the cholesterol content of the blood fluid is responsible for the great increase in resistance of the red cells against sapotoxin solutions.

TABLE VI

JAUNDICE AND RED CELL RESISTANCE TO SAPOTOXIN SOLUTIONS

TYPE	CASES	RESISTANCE	1:13,000	HB.	VARIATION IN RESISTANCE
Normals	185	1:13,937	10.7	91	: - - - - -
All Types	31	1:12,685	55.0	79	+ 1,252
Obstructive	15	1:10,033	?	84	+ 3,904
Hemolytic	5	1:18,600	6.8	81	- 4,663
General	11	1:13,182	?		+ 755
Cirrhosis	4	1:14,500	15.8	77	- 563

TABLE VII

RELATION OF THE BLOOD FLUIDS TO RESISTANCE OF THE RED CELLS

EXP. NO.	RESISTANCE OF CELLS IN WHOLE BLOOD	WASHED CORPUSCLES DILUTED 1:1 WITH NaCl SOLUTION	WASHED CORPUSCLES DILUTED 1:1 WITH OWN SERUM	WASHED CORPUSCLES DILUTED 1:1 WITH SERUM OF OPPOSITE BLOOD	CHOLESTEROL
X.	1:12,750	1:38,000	1:13,750	1:10,750	166
Y.	1: 9,000	1:34,000	1:11,500	1:17,500	292
Difference	3,750	4,000	2,250	6,750	126

Case X was suffering from a slight attack of gastritis.
Case Y was suffering from a severe attack of obstructive jaundice.

VIII. ANEMIA AND THE RESISTANCE OF THE RED BLOOD CELLS

The lowered resistance of the red blood cells in pernicious anemia has been appreciated for a long time. In other forms of anemia conflicting reports have appeared relative to the red cell resistance. In secondary anemias the resistance to salt solutions is raised. "Anemia" in itself means only that the number of red cells per given volume or their hemoglobin content is decreased. The causes of anemia are many, hence, the factors giving rise to this condition are of more importance from the clinical standpoint than the mere reduction in the cell count or the degree of hemoglobin. Anemias are associated with many diseases and their causative factors, it seems, should determine the blood cell reactions to hemolytic agents. Furthermore, the chemistry of the blood in cases of anemia determine the degree of resistance to laking agents, for the red cells must of necessity remain approximately

normal in order to exist as cells. Also, a process of cell destruction is continually at work in the normal blood stream, hence alterations in the blood chemistry may permit the hemolytic agents normally present unopposed play upon the red cells resulting in their untimely destruction. Oleic acid in excess in the blood stream acts as a powerful hemolytic agent. A continued action of such an acid must of necessity result in an anemia.

The work on the cholesterol content of the blood seems to throw some light on the probable cause of the various degrees of resistance shown by the red cells in different types of anemia. In pernicious anemia there is a definite reduction in the cholesterol content of the blood. Dennis[12] reports that the removal of the spleen results in an increased cholesterol content of the blood in every case, but that there is no change in the fat content of the blood. The excellent works of Bloor[3] have shown that the lipoid value of the blood in anemia is normal as long as the percentage of blood corpuscles remains above one-half the normal number per volume. Whenever the percentage drops below this level abnormalities appear, which in the order of their magnitude and occurrence are: (1) high fat in plasma; (2) low cholesterol in plasma and occasionally in the corpuscles; (3) low lecithin in the plasma. These results, it is true, offer no certain evidence that abnormalities in the blood lipoids are responsible for anemia, however, the low value for cholesterol which is an antihemolytic substance, and the high fat fraction which may indicate the presence of abnormal amounts of hemolytic lipoids in the blood may be considered as possible causative factors in the production of anemia. Such argument is supported by the increase in cholesterol of the blood following splenectomy in cases of anemia.

Inasmuch as cholesterol is known to act as an antihemolytic agent against sapotoxin, the varying degrees of resistance offered by the red blood cells in cases of anemia may be assumed to depend upon the degree of protection offered by the serum to the cells during the presence of a hemolytic agent. Such a suggestion seems probable in the light of our present results.

Cases showing a reduction in the red cell count demonstrated varying degrees of resistance to laking agents. The average resistance of 43 such cases demonstrated a resistance to sapotoxin of 1:13,889, practically normal. The average red cell count was 3,022,553. The average hemoglobin was 55 per cent.

TABLE VIII

ANEMIA AND THE RESISTANCE OF THE RED BLOOD CELLS TO SAPOTOXIN

ANEMIA	CASES	RESISTANCE	1:13,000	HB.	RED COUNT
Pernicious	8	1:15,063	8.0	35	1,135,000
Cardiorenal	8	1:12,312	8.5	60	2,570,875
Syphilis	7	1:15,750	10.4	58	3,936,000
Hemolytic Jaundice	4	1:17,125	7.7	58	3,050,000
Carcinoma	5	1:12,190	17.3	51	2,796,000
Tuberculosis	4	1:13,250	13.4	69	4,263,333
Undetermined	7	1:13,040	16.0	58	3,406,666
All cases	43	1:13,889		55	3,022,553
Normals	185	1:13,937	10.7	91	Not determined

Anemias seem to divide into three types, those with a high resistance, those with practically normal resistance and those with low resistance (Table VIII).

The average resistance of 8 cases of pernicious anemia was 1:15,063, or a reduction from the average normal of 1,126 points. Eight cases of anemia associated with cardiorenal diseases gave an average of 1:12,312; a resistance 2,751 points greater than for pernicious anemia. The average resistance of 7 luetic cases was 1:15,750 or a reduction of 1,813 points below the average normal. Four cases of hemolytic jaundice with low red cell counts gave an average resistance to sapotoxin of 1:17,125 or a reduction of 3,188 points below the average normal. Five carcinoma cases with low cell counts gave an average resistance of 1:12,190 or an increased resistance of 1,747 points over the average normal. Tuberculous patients (4) showing low cell counts gave an average resistance to sapotoxin solutions of 1:13,250 or 687 points greater than the normal resistance. The remaining 9 cases showed a reduction in the red cell count from undetermined and various causes. The average resistance for these cases was 1:13,040 or an increase over the average normal of 897 points.

IX. CARDIORENAL DISTURBANCES AND RED CELL RESISTANCE

Arterial hypertension is often accompanied by an increased resistance of the red cells. A similar increased resistance has also been reported for nephritis and diabetes. In eclampsia the resistance is markedly increased to anisotonic salt solutions and to saponins.

Cholesterol remains normal or is increased in nephritis (Gorham[13]), however, cholesterol combined as esters is low. Bloor[3] has found that in severe nephritis there is a high content of fat in the plasma and corpuscles, and an increased amount of lecithin in the corpuscles. The cholesterol content remains essentially normal. Similar conditions are found in alimentary lipemia. Inasmuch as an alkalinity of the blood and tissues is necessary for their normal functioning, it seems probable that the relation of fat assimilation found in nephritis is one manifestation of a general phenomenon brought about by a decreased blood and tissue alkalinity. The observations of De Langen are of interest.[11] He reports that the Javanese peoples show about one half the cholesterol value of the blood as do the Europeans. Gall bladder trouble, diabetes and chronic nephritis is rare among these peoples.

Twelve cases of nephritis gave an average resistance of 1:13,958 (Table IX). The average hemoglobin was 79 per cent. The average findings for chemical analyses of the blood of these patients was urea nitrogen 76.8, uric acid 6.21, creatinin 5.30, sugar 0.127 and carbon dioxide 30.7.

Five cardionephritic cases gave an average resistance of 1:14,500 or a reduction of 563 points below the normal average. The chemical analysis of the blood of these patients was urea nitrogen 24.5, uric acid 3.50, creatinin 1.82, sugar 0.156 and carbon dioxide 55.0.

Two cases of diabetic coma averaged a resistance of 1:13,500. The hemoglobin was 88 per cent and blood sugar 1.700.

TABLE IX

KIDNEY DISEASES AND THE RESISTANCE OF THE RED CELLS TO SAPOTOXIN

DISEASE	CASES	RESISTANCE	1:13,000	HB.	RESISTANCE VARIATION
Nephritis	12	1:13,958	17.5	79	Normal
Cardionephritis	5	1:14,500	10.3	84	−563 points
Eclampsia	2	1:10,500	20.4	83	+3,437 points
Uremia	5	1:14,050	16.7	85	Normal
Diabetes	2	1:13,500	14.0	88	+437 points
Calcium Chloride*	15	1:13,950	--	90	Normal
Ammonium Chloride*	15	1:13,317	--	90	+620 points
Urea*	6	1:12,666	24.6	90	+1,271 points
Normals	185	1:13,937	19.7	91	-----

*Average of all determinations while on drug.

Five cases of uremia gave an average resistance of 1:14,050. The hemoglobin averaged 85 per cent. Chemical analyses of the blood showed a urea nitrogen content of 16.5 and carbon dioxide of 58.

Two cases of eclampsia demonstrated a resistance of 1:10,500 or an increase in the red cell resistance of 3,437 points above the average normal.

The addition of 1 to 3 minims of a 1 per cent solution of urea causes the resistance of normal red cells to become increased. Three determinations on the normal blood gave an average resistance of 1:14,000, hemoglobin 90; time for complete hemolysis to occur in a 1:13,000 solution 12 minutes. The average resistance of 6 determinations to which urea solutions had been applied was a resistance of 1:12,666; complete hemolysis 24.6 minutes; hemoglobin 90 per cent.

The effect of calcium and ammonium chloride upon red cell resistance was determined in 15 cases each (Table IX). The average resistance of the red cells in the calcium experiment was 1:13,950. The average hemoglobin was 90. The first day following the taking of 60 grains of calcium chloride the resistance was 1:13,300; the second day 1:13,700 and the third day 1:14,100. Calcium chloride, therefore has little or no effect upon the resistance of the red cells to sapotoxin solutions. The average of 15 cases was 1:12,900. The first day following the ingestion of 45 grains of ammonium chloride resulted in an increased resistance of 1,037 points above the normal average. The second day the resistance was 1:13,000, an increase of 937 points and the third, 1:14,900. Therefore, ammonium chloride causes an increase in the resistance of the red blood cells immediately following its administration, however, resistance is not permanently influenced by ammonia, for on the third day the resistance is back to normal.

X. MALIGNANT GROWTHS AND THE RESISTANCE OF THE RED BLOOD CELLS

The resistance of the red cells may or may not be reduced in cases of malignancy.

Gorham and Myers[13] state that a condition of hypocholesterolemia is found in the cachexia of malignancy and all anemias of the pernicious type. Bloor and Knudson[3] have shown that in normal blood there is a constant re-

lation between the free cholesterol and cholesterol esters. In whole blood the average percentage of cholesterol in combination as esters is about 33.5 per cent, in plasma 58 per cent of the total cholesterols. In pathologic conditions the relation between free and bound cholesterol remains normal in all cases except carcinoma and most cases of nephritis, in both of which the per cent combined as esters is low. Luden[14] has shown that the the cholesterol content of the blood may be increased by diet also that a cholesterol increase results in a coincident weakening of the lymphoid defense. Hence, a decrease in the lymphoid defense accompanied by an increase in cholesterol may act as a predisposing factor in the production of cancer.

The resistance of red blood cells in 19 cases of malignant growths of various parts of the body gave an average resistance of 1:13,079. This represents an increased resistance of 858 points above the normal average. The average time required for complete laking in a 1:13,000 solution was 16 minutes. The greatest individual resistance (Case No. 890) 1:9,000 was found associated with a carcinomatous gall bladder. The lowest resistance—1:14,500—(Case No. 537) was shown by one suffering from visceral growths and syphilis. The average hemoglobin of the 19 cases studied was 78 per cent. Hence, there

TABLE X

MALIGNANT GROWTHS AND RED CELL RESISTANCE

	CASES	RESISTANCE	1:13,000	HB.
	19	1:13,079	16.0	78
Normals	185	1:13,937	10.7	91
Difference		+ 858	5.3	13

is a slight increase in the resistance of the red blood cells in cases of malignant growths. However there is no constancy in the determinations; other pathologic conditions greatly alter the results. However, the early cases of carcinoma did show a higher degree of resistance in the red cells than in those cases associated with anemia or marked cachexia (Table X).

SUMMARY AND CONCLUSIONS

I. Pregnancy is associated with an increased resistance of the red cells to sapotoxin. At the time of delivery the blood cells show their greatest or maximal resistance to the action of sapotoxin. Immediately following parturition the resistance of the erythrocytes begins to diminish and upon the 12th day has materially returned toward the normal. Hemoglobin determinations vary but slightly during the latter part of pregnancy and immediately following parturition. The increase in blood cholesterol, may in part account for the rise in red cell resistance to sapotoxin.

II. Malaria as such does not appear to lower the resistance of the red blood corpuscles to sapotoxin. The administration of quinine causes a decrease in the resistance, this decrease lasting throughout the period of quinine administration. Nonmalarial cases fail to show any constant deviation from the normal average resistance either during the taking of quinine or following its withdrawal. Hence the loss of resistance on the part of malarial blood following the administration of quinine is characteristic of malaria plus quinine.

That is, quinine appears to act in usch a manner as to bring about a weakened resistance in malarial cells. Therefore the assumption may be made that quinine aids in the destruction of the red cells carrying the plasmodium of malaria. The low cholesterol content of the blood also predisposes to the hemolytic substances present during malaria.

III. Pulmonary Tuberculosis tends to increase the resistance of the red blood cells to the hemolytic action of sapotoxin. Pregnancy in such cases accentuates the effects of tuberculosis. Tuberculosis complicated with syphilis tends to bring about a depression in the resistance of the red cells.

IV. Typhoid Fever appears to alter but little the resistance of the red cells.

V. In Syphilis—positive Wassermann—the resistance of the red cells is less than that for the average normal. The administration of mercury alone tends to increase the resistance. The addition of iodides to the mercurial treatment causes a reduction in the resistance of the red cells for several days; the effects cannot be maintained. Luetic patients on iodides alone show but little change in the resistance of the red cells.

VI. Lead Poisoning may bring about an increased resistance of the red cells to sapotoxin solutions.

VII. Jaundice and blood cell resistance appear to bear no constant relationship, that is, jaundice as such is in no way an indication of blood cell resistance. Obstructive jaundice causes a marked increase in the resistance of the blood cells, on the other hand, hemolytic jaundice is associated with a marked decrease in the resistance of the erythrocytes. Other types of jaundice may or may not be associated with variations in the resistance of the red cells. The relation of blood cholesterol to red cell resistance is such as to lead to the assumption that cholesterol is a depressant to the hemolytic action of sapotoxin.

VIII. Anemia associated with lues and hemolytic jaundice shows a lessened degree of resistance of the red cells to sapotoxin. The red cells in anemias associated with or caused by carcinoma, tuberculosis, obstructive jaundice and some cases of pernicious anemia show an increased resistance towards hemolytic agents. Anemia as such does not appear to affect the red cell resistance, something more than a mere reduction in the number of cells determines the degree of the resisting power of the red blood cells.

IX. Cardiorenal Diseases. Arteriosclerosis is associated usually with an increased resistance of the red cells to sapotoxin. Diabetic coma and uremic conditions in general are not associated with marked alterations of the red cell resistance. Cases diagnosed as nephritis give normal resistance reactions, however, cases diagnosed as cardionephritics seem to show some slight degree of reduction of resistance. The addition of urea solutions to red cells tends to increase their resistance. Calcium chloride administered by mouth does not affect the red cell resistance. The taking of ammonium chloride by mouth tends to increase the resistance of the cells for a short period only. The resistance returns to normal even during the administration of the drug. The relation of cholesterol in the blood in cardionephritic cases to the resistance of the red cells is striking. The increased lecithin in the corpuscles of severe nephritis, however, leads one to suspicion the validity of this relation. How-

ever, the increased cholesterol of the serum may be sufficient to protect the cells in spite of the increased lecithin content.

X. Malignant Growths as such do not appear to alter the red cell resistance; there is no constancy in the determinations. However, the early cases of carcinoma show a slightly higher resistance than cases associated with anemia or cachexia.

XI. The Cholesterol content of the blood appears to run parallel to the degree of resistance of the red cells to sapotoxin solutions. A high cholesterol content of the blood is associated with a high degree of resistance of the red cells when they are surrounded with serum. A low cholesterol content is associated with a reduced resistance of the cells.

XII. Therefore, the resistance of the red cells to sapotoxin solutions varies in different diseases and this variation is intimately associated with alterations in the metabolism of cholesterol.

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LABORATORY METHODS

A SYSTEM OF LABORATORY EXAMINATIONS AND RECORDS*

By John A. Kolmer, M.D., Philadelphia, Pa.

THE following system of making and filing requests and reports for laboratory examinations has been gradually constructed and found uniformly satisfactory in the wards and dispensaries of the Polyclinic and Medico-Chirurgical hospitals of the Graduate School of Medicine of the University of Pennsylvania.

The problem of constructing an efficient system is frequently troublesome and probably no single system will meet all conditions in individual institutions; the essential requirements which I have had in mind in piecing together the present system have been as follows:

1. A means for making requests for laboratory examinations in writing with the minimum of time and work. This condition has been met by adopting a request card devised by Dr. C. Y. White, which the physician can fill out in a few minutes.

2. A means for furnishing the laboratory with some clinical data in the interests of more intelligent work and cooperation. This condition is fulfilled by the same request card.

3. A means for returning a report to the hospital or out-patient clinic and making a duplicate for filing in the laboratory with the minimum of time and work.

4. A means for recording the results of laboratory examinations on the history of each patient in such manner as permits binding and avoids too much bulk.

5. A system for filing and recording duplicate reports in the laboratory readily accessible and facilitating the compilation of weekly, monthly or yearly reports with a minimum of work and trouble.

6. A means for providing each physician with a record of laboratory examinations which he may use for his private records.

The system herein described has met these essential requirements and can be readily modified if necessary to suit individual conditions.

THE REQUEST CARD

In most institutions requests for laboratory examinations are made on the upper half of printed blanks of paper of the size of history sheets which are filled out with the name of the patient, ward and such essential data and for-

*From the Department of Medical Sciences of the Medico-Chirurgical Graduate School of Medicine of the University of Pennsylvania.

Lab. No. _____ Rec. at Lab. _____

Request for Laboratory Examination Report Returned _____

(Underscore Specimen and Examination Requested. Fill out properly.)

Name **Age** **Date**

Request of Dr. **Ward—Clinic—Room**

Specimen of: Urine (for special only), Blood, Sputum, Gastric Contents, Feces, Tissue, Pus, Fluid, Culture

For: Chemical, Microscopical, Bacteriological, Serological, Pathological Examination

From No. of Specimens accomp. card

Clinical Diagnosis:

Clinical Data

Nature of Examination Requested

Laboratory Report:

Dr. Dr.

Res. Phys. Assist. Phys. Physician

THIS HALF OF CARD WILL BE FILED IN THE LABORATORY

Laboratory Report Received: _____

(Copy Report on History) Returned: _____

Name **Date** **Lab. No.**

Report to Dr. **Ward—Clinic—Room**

Specimen of: Urine (for special only), Blood, Sputum, Gastric Contents, Feces, Tissue, Pus, Fluid, Culture

For: Chemical, Microscopical, Bacteriological, Serological, Pathological Examination

From No. of Specimens received

Laboratory Report (Results of Blood Examination on other side)

Examined by Dr. and Dr.

REPORT CONTINUED ON OTHER SIDE

Fig. 1.—Front of request card.

Blood Report		Report Continued
Erythrocytes		General Description of Specimen, when received
Leucocytes		
Hemoglobin		
Color Index		
Differential		
Small Lymph.		
Large Lymph.		
Transitionals		
Polymorpho.		
Eosinophiles		
Basophiles		
Abnormal Erythrocytes		
Abnormal Leucocytes		

Blood Report		Report Continued
Erythrocytes		General Description of Specimen, when received
Leucocytes		
Hemoglobin		
Color Index		
Differential		
Small Lymph.		
Large Lymph.		
Transitionals		
Polymorpho.		
Eosinophiles		
Basophiles		
Abnormal Erythrocytes		
Abnormal Leucocytes		

Fig. 2.—Back of request card.

warded to the laboratory with the specimen; the laboratory report is written on the lower half of the blank and the whole sheet returned to the hospital.

The usual objections to this system are: (a) the sheet may become soiled and creased; (b) when a large number of examinations are made an accumulation of these blanks renders the history too bulky for binding and (c) these blanks usually fail to furnish the pathologist with some clinical data. This system is particularly apt to be unsatisfactory in out-patient departments unless the laboratory reports are transcribed to the history card.

The request blank shown in Figs. 1 and 2 is slightly modified from that

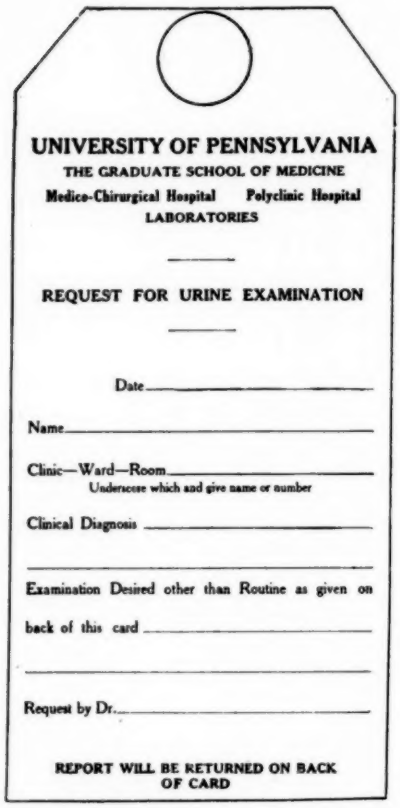


Fig. 3.—Front of card used for routine urine examinations.

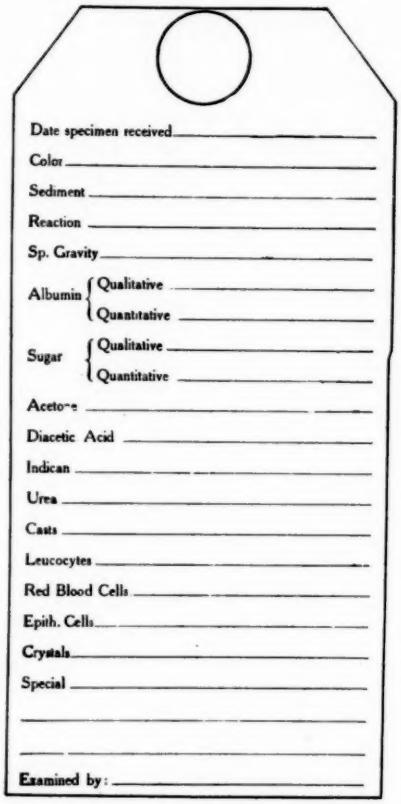


Fig. 4.—Back of card used for routine urine examinations.

devised by Dr. C. Y. White of Philadelphia and used in the Episcopal hospital; it has been found very serviceable and satisfactory.

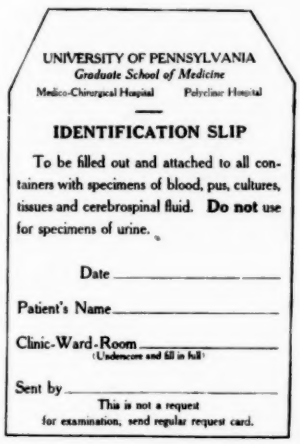
The blank is printed on thin cardboard, measures 4 by 12 inches and is perforated in the middle so that it may be divided into two cards.

Fig. 1 shows the front and Fig. 2 the back of this card.

The physician fills out the left-hand portion of the card (Fig. 1) which provides space for the name of the patient, age, ward, date and name of attending physician; simply drawing a line under the nature of the specimen and the kind or kinds of examinations requested and signing completes the essentials. Space is provided for the clinical diagnosis (usually provisional)

and also for further details of the examination requested, if the latter is necessary. Space is also provided for the chief clinical data bearing upon the laboratory examination; for example, the kind and duration of treatment in relation to a request for the Wassermann test. *Only the first request card for a patient need be filled out with the details of diagnosis and clinical data;* when subsequent examinations are requested the essential data alone is sufficient (name of patient and physician, date, ward and examination requested). Dr. White uses a green card for the first request and white cards for subsequent requests. I have found this a good scheme but likely to produce confusion and a great deal of trouble in insisting upon the proper use of the two cards; for these reasons I have abandoned the use of the green card as being nonessential.

A clerk in the laboratory records on each half of the card the date and hour received and subsequently the date when the report is returned (which are frequently the means for settling a dispute regarding a delay in returning a report due to delay in forwarding the request or specimen or both).


UNIVERSITY OF PENNSYLVANIA
Graduate School of Medicine
Medico-Chirurgical Hospital Polyclinic Hospital

IDENTIFICATION SLIP

To be filled out and attached to all containers with specimens of blood, pus, cultures, tissues and cerebrospinal fluid. **Do not** use for specimens of urine.

Date _____

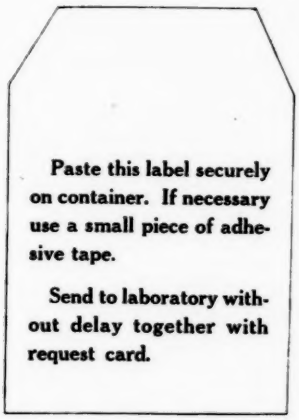
Patient's Name _____

Clinic-Ward-Room _____
(Underscore and fill in full)

Sent by _____

This is not a request for examination, send regular request card.

Fig. 5.—Front of the identification tag attached to specimens.


Paste this label securely on container. If necessary use a small piece of adhesive tape.

Send to laboratory without delay together with request card.

Fig. 6.—Back of the identification tag attached to specimens.

The right-hand half of the card is filled out in the laboratory although some physicians are willing to give an extra minute or two for this; *the laboratory report is returned to the hospital on this half of the card.* The pathologist also writes a synopsis of his report on the left-hand portion of the card.

The left-hand half of the card is filed in the laboratory (alphabetically); the right-hand half is returned to the hospital. A cross index may be kept in the laboratory for filing the necropsies, examination of tissues, blood, sputa, etc.

The results of blood examinations are filled in on the back of each card, which also provides additional space for other laboratory reports.

As a general rule only one kind of an examination is requested on a card. For example, a general blood examination including erythrocyte and leucocyte counts, differential leucocyte count, hemoglobin estimation and color index may be requested on one card but it is not permissible in these laboratories to request on one card for example, a general blood examination and an examination of sputum or some other examination; a second card would be required

for an examination of the sputum. The main reasons for this are to prevent overcrowding a card with the necessary laboratory reports and to facilitate filing a laboratory record.

This card is not used for requests for routine urine examinations; a special card is provided shown in Figs. 3 and 4, which is tied to the bottle or container and the report returned on the back of the same card (Fig. 4).

Dr. John Eiman, pathologist to the Presbyterian Hospital of Philadelphia, has suggested the use of rubber stamps in the laboratory for stamping the cards in making out reports; I use the following list and find them very useful for saving time and overcoming the difficulties of poor and illegible writing:

BLOOD EXAMINATIONS

Anisocytosis	Polychromatophilia
Poikilocytes	Basophilic degen.
Macrocytes	Myelocytes
Microcytes	Coagulation Time
Macroblasts	Malaria
Normoblasts	Platelets
Microblasts	Culture

GASTRIC ANALYSIS

Amount	Total Acidity
Appearance	Free HCl
Odor	Comb. HCl
Mucus	Acid Salts
Blood	Lactic Acid
Microscopical:	Fractional Analysis:
	Total Acidity
	Free HCl
	Protein

PUNCTURE FLUIDS

Amount	Color
Appearance	Specific Gravity
Reaction	Sugar
Proteins	
Cells per 1 c. mm	
Polys %	Lymph % Endoth %
R. B. C.	Animal Inoculation
Smears and Cultures	
Colloidal Gold	

FECES EXAMINATION

Form	Bile
Consistency	Reaction
Color	Curds
Mucus	Concretions
Blood	Parasites
Pus	Ova
Microscopical:	Bacteriological:

SPUTUM EXAMINATIONS

Quantity	Blood and Pus
Appearance	Tubercle Bacilli
Color	Fungi
Cells	Pneumococci-Type

BACTERIOLOGICAL

Smear of	Result
Culture of	Result

WIDAL REACTION

Time
Dilutions
Results

Specimens of tissue, feces, sputa, etc., may be labeled with adhesive bands bearing the name of the patient; I have used the identification tag shown in Figs. 5 and 6 with entire satisfaction.

In this institution the specimens and cards are carried by the nurses or orderlies to a specimen room set aside in each hospital; at 9 and 11 A. M. and 3 P. M. a laboratory orderly collects these and brings them to the laboratories. This system prevents confusion and tends to fix responsibility for delivery of request cards and specimens.

Urgent examinations are made at any time, the physician marking the card "rush" and sending it at once to the laboratories by a nurse or orderly; these requests receive instant attention.

In exceptional instances telephone requests are accepted *but are always followed up by the request card properly filled in* with the necessary data.

Requests for necropsies are made out on the same card (the legal permits being kept and filed in the office of the hospital); necropsy reports are returned on a set of blanks modified and enlarged by Dr. White from those originally devised by the late Dr. A. O. J. Kelly. These printed blanks are unusually complete and when properly filled out furnish a splendid record of a necropsy which is easily bound with the clinical history.

All laboratory examinations are made as far as possible on the same day as when the requests and specimens are received; the reports are returned to the respective wards and out-patient departments of the hospitals once or twice daily by the resident physicians serving in the laboratories, this system definitely fixing responsibility for the prompt and safe delivery of reports.

RECORDING LABORATORY REPORTS ON HISTORY

When the reports are returned to the hospital they are copied by the resident physicians, nurses, or a special clerk to the histories of the patients. The necessity for copying constitutes the single weak point in this system by reason of the opportunity for error in copying the reports or individual carelessness. As a matter of experience, however, errors rarely occur and none have occurred since rubber stamps have been used as described above in making out laboratory reports, which largely eliminates bad writing.

Three blanks are provided for these reports shown in Figs. 7, 8 and 9; each sheet measures 9½ x 12 inches and is bound with the rest of the history.

The first sheet (Fig. 7) provides space for recording seven routine and three special examinations of blood and urine. If only urine and blood examinations have been requested, this would be the only sheet required.

The second sheet (Fig. 8) provides for recording examinations of stomach contents and vomitas, feces, sputum and serological reactions. This sheet is attached to the history only in case any of these examinations have been made in order to avoid the waste of binding a blank.

The third sheet (Fig. 9) provides space for recording the results of examinations of cerebrospinal fluid, pus, transudates, cyst fluids and secretions; also general bacteriological examinations, examination of tissues and special chemical examinations of blood and urine.

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Polyclinic Hospital

Name_____
Case No._____

LABORATORY REPORTS (1)

GENERAL URINE EXAMINATIONS

DATE						
Quantity						
Color						
Sediment						
Reaction						
Sp. Gravity						
Albumin (Qualitative)						
Albumin (Quantitative)						
Sugar (Qualitative)						
Sugar (Quantitative)						
Acetone						
Diacetic Acid						
B-Oxybutyric						
Indican						
Urea						
Bile						
Total Nitrogen						
Microscopical						
Casts						
Leucocytes and Pus						
Red Blood Corpuscles						
Epith. Cells						
Cylindroids						
Mucus						
Crystals						
Special						
Examiner						

SPECIAL URINE EXAMINATIONS

DATE			
Functional Kidney Test			
Bacteriological			
Animal Inoculation for T. B.			
Special Chemical Exam.			
Examiner			

GENERAL BLOOD EXAMINATIONS

DATE						
Erythrocytes						
Leucocytes						
Hemoglobin						
Color Index						
Differential						
Small Lymph.						
Large Lymph.						
Transitionals						
Polymorph.						
Eosinophiles						
Basophiles						
Abnormal Erythrocytes						
Abnormal Leucocytes						
Examiner						

SPECIAL BLOOD EXAMINATIONS

DATE			
Blood Culture			
Coagulation Time			
Malaria			
Basophilic Degeneration			
Blood Platelets			
Parasites			
Special Chemical (Urea, Sugar, etc.)			
Examiner			

Fig. 7.—Sheet used for recording results of laboratory examinations; bound with the history.

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Polyclinic Hospital

Name_____

Case No._____

LABORATORY REPORTS (2)

SEROLOGICAL EXAMINATIONS

DATE			
Wassermann			
Gono. Comple. Fixation			
Widal			
Special			
Examiner			

GASTRIC ANALYSIS (INCLUDING VOMITUS)

DATE			
Amount			
Gross Appearance			
Odor			
Total Acidity			
Free Hydrochloric Acid			
Combined Acids			
Acid Salts			
Lactic Acid			
Blood			
Ferments			
Fractional Analysis			
Total Acidity			
Free Hydrochloric Acid			
Protein			
Microscopical Examination			
Special			
Examiner			

GENERAL FECES EXAMINATIONS

DATE			
Form			
Consistency			
Color			
Mucus			
Blood			
Pus			
Bile			
Reaction			
Curds			
Concretions			
Parasites			
Ova			
Microscopical			
Special			
Examiner			

SPECIAL FECES EXAMINATIONS

DATE			
Cultures—Bacteriological			
Animal Inoculation for T. B.			
Examiner			

SPUTUM EXAMINATIONS

DATE					
Quantity					
Character—Gross Appearance					
Color					
Microscopical					
Cells					
Fungi					
Blood and Pus					
Bacteriological					
Tubercle Bacilli					
Pneumococci—Type					
Special					
Examiner					

Fig. 8.—Sheet used for recording results of laboratory examinations; bound with the history.

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Polyclinic Hospital

Name_____

Case No._____

LABORATORY REPORTS (3)

CEREBROSPINAL FLUID, PUS, TRANSUDATES, CYST FLUID AND SECRETIONS

DATE				
Amount				
Gross Appearance				
Color				
Sp. Gravity				
Reaction				
Pressure				
Cells per Cubic Millimeter				
Kinds of Cells				
Lymphocytes				
Endothelial				
Polymorphonuclears				
Red Blood Corpuscles				
Protein				
Sugar				
Smears				
Cultures				
Animal Inoculation				
Wassermann Reaction				
Colloidal Gold Reaction				
Special				
Examiner				

GENERAL BACTERIOLOGICAL EXAMINATIONS

DATE				
Vaginal Smears and Washings				
Smears (mention source)				
Cultures of Throat and Nose				
Cultures of Other Parts (mention)				
Special				
Examiner				

EXAMINATION OF TISSUES REMOVED AT OPERATION

DATE	EXAMINER

SPECIAL CHEMICAL EXAMINATIONS OF BLOOD AND URINE

DATE				
Sugar				
Creatinine				
Creatine				
Uric Acid				
Urea				
Non Protein Nitrogen				
Chlorides				
Carbon Dioxide Combining Power				
Total Nitrogen				
Total Solids				
Examiner				

Fig. 9.—Sheet used for recording results of laboratory examinations; bound with the history.

Similar blanks are used for recording laboratory reports in the out-patient departments inasmuch as the history blanks are of the same size as used in the hospitals.

PROVIDING SEPARATE REPORTS FOR THE ATTENDING PHYSICIAN

When a report has been copied from a card to the history as described, the latter has fulfilled its function and may be discarded. It is marked "received and copied" and attached temporarily to the history board where the attending physician may obtain it, "post-officed" in the physicians' room of the hospital or mailed direct to the physician; by any of these methods the physician may obtain the report for his private records.

This is a matter of considerable importance and convenience when the patient leaves the hospital before the laboratory examinations are completed and the reports returned; under these circumstances the reports are taken to the office of the hospital and copied on the history but the physician may never see the report. With any of the above plans in operation the attending physician is bound to receive the report and experience has taught that *no system is successful unless it literally forces the report upon the attention of the physician with the minimum of effort on the part of the latter.*

LABORATORY RECORDS

When a request card is received in the laboratory by a clerk it is recorded, given a serial number and the date and hour written on both halves of the card (upper right-hand corners) as shown in Fig. 1. *All request cards and specimens are delivered to a single designated place in the laboratory so that all pass through the hands of a clerk for purposes of record.* Even "rush" requests are first recorded, although in case of unavoidable delay in recording the card the examination may proceed and the record be made afterward.

The main purpose of recording each request card is to guard against loss and provide a means for tracing a card in case of a miscarriage. For example, a request card may miscarry in the laboratory after being recorded.

For the purpose of recording the requests for laboratory examinations, seven cards of *different colors* are employed. Each card measures six by nine inches, is ruled on both sides and provides from forty to fifty entries.

Fig. 10 shows the card used for recording requests for blood examinations; this card is colored red.

Fig. 11 shows the card for feces examinations (brown).

Fig. 12 is the card for examination of stomach contents and vomitus (pea green).

Fig. 13 is the card for sputum examinations (gray).

Fig. 14 is the card for examinations of cerebrospinal fluid, pus, transudates, cyst fluid and secretions (white).

Fig. 15 is the card for general bacteriologic examinations (yellow).

Fig. 16 is for recording requests for tissue examinations and necropsies (pink).

Fig. 17 is the card for recording special urine examinations (yellow). Routine urine examinations are not recorded separately; simply the total number

examined each day is recorded on a card or in a book. Duplicate records of the results of ordinary or routine urine examinations are not kept, mainly because the large amount of time required for recording fifty or more examinations per day is not warranted by the value of the records.

When a request card is received the clerk gives it a serial number, writes into the appropriate laboratory record the date when received, the name of the

RECORD OF BLOOD EXAMINATIONS

LAB. No.	DATE REC'D	REQUEST OF DR.	R.B.C's	W.B.C's	HB.	DIFF.	WASS.	GONO.	CULT.	WIDAL	COAG.	Malaria	Platelets	Special	CHEMICAL	EXAM. BY DR.	DATE RTD.
DATE CARD BEGAN DATE CARD FILLED		GRAND TOTALS															

Fig. 10.—Card (red) used for filing requests for blood examinations.

RECORD OF FECES EXAMINATIONS

LAB. No.	DATE REC'D	REQUEST OF DR.	PHYS	OVA	PARASITES	CONCRETIONS	BLOOD	FOODS	PUS	CURDS	BILE	CULTURE	FOR T. B.	SPECIAL	EXAM. BY DR.	DATE RTD.
DATE CARD BEGAN DATE CARD FILLED		GRAND TOTALS														

Fig. 11.—Card (brown) used for filing requests for feces examinations.

physician making the request, the name of the examiner in the laboratory and checks off the kind or kinds of examinations requested.

The number of any kind of examination or examinations can be seen at a glance and at the end of a week, month or year a detailed laboratory report or summary can be prepared by a clerk with a minimum of time and work by simply summarizing these cards. The system has proved eminently satisfactory,

RECORD OF STOMACH CONTENTS AND VOMITUS EXAMINATIONS

LAB. No.	DATE REC'D	REQUEST OF DR.	CHEM.	MICRO.	BAC-TERIOL.	BLOOD	BILE	LACTIC ACID	PROTEIN	FRAC-TIONAL	SPECIAL	EXAM. BY DR.	DATE RTD.
DATE CARD BEGAN: DATE CARD FILLED:		GRAND TOTALS											

Fig. 12.—Card (pea green) used for filing requests for examination of stomach contents.

RECORD OF SPUTUM EXAMINATIONS

LAB. No.	DATE REC'D	REQUEST OF DR.	PHYS.	FOR T. B.	TYPE PNEU.	FOR PNEU.	PROTEIN	PUS	BLOOD	ELASTIC	SPIRALS	SPECIAL	EXAM. BY DR.	DATE RTD
DATE CARD BEGAN: DATE CARD FILLED:		GRAND TOTALS												

Fig. 13.—Card (gray) used for filing requests for sputum examinations.

being simple, accurate, and inexpensive. By employing the seven different cards identified at a glance by their colors and dividing laboratory examinations into these seven main groups, it is a simple matter indeed to look up a record.

As previously stated the right-hand half of each request card is retained in the laboratory and filed alphabetically in cabinets. This provides a complete record including a complete report of the laboratory examinations. *In case*

RECORD OF CEREBROSPINAL FLUID, PUS, TRANSUDATES, CYST FLUID AND SECRETIONS EXAMINATIONS

LAB. No.	DATE REC'D	REQUEST OF DR.	PHYS.	BAC-TERIOL.	FOR T. B.	TOTAL CELLS	KIND CELLS	PROTEIN	SUGAR	WASS.	GOLD	SPECIAL	EXAM. BY DR.	DATE RTD.
DATE CARD BEGAN: DATE CARD FILLED:		GRAND TOTALS												

Fig. 14.—Card (white) used for filing requests for examination of cerebrospinal fluid, pus, transudates and exudates.

RECORD OF GENERAL BACTERIOLOGICAL EXAMINATIONS

LAB. No.	DATE REC'D	REQUEST OF DR.	VAGINAL	EYE	EAR	NOSE	THROAT	SKIN	TISSUE	VACCINE	SPECIAL	EXAM. BY DR.	DATE RTD.
DATE CARD BEGAN: DATE CARD FILLED:		GRAND TOTALS											

Fig. 15.—Card (yellow) used for filing requests for bacteriologic examinations.

a report miscarries and is lost in the hospital before being recorded on the history, it is a simple matter to furnish a duplicate report from the laboratory files; this feature of the system has proved very valuable and especially for the out-patient departments, where laboratory reports are more apt to be misplaced or lost than in the wards and private rooms.

The laboratory can extend this system by any internal arrangement of cross indexes; the above system embraces the essentials.

RECORD OF TISSUE EXAMINATIONS AND NECROPSIES

LAB No	DATE REC'D	REQUEST OF DR.	SURG.	GYN.	EYE	NOSE	THROAT	SKIN	SPECIAL	EXAM BY DR	DATE RTD
DATE CARD BEGAN: DATE CARD FILLED:		GRAND TOTALS									

Fig. 16.—Card (pink) used for filing requests for tissue examinations and necropsies.

SPECIAL URINE EXAMINATIONS

LAB. No.	DATE REC'D	REQUEST OF DR.	CULT.	FOR T. B.	ANIMAL TEST FOR T. B.	FUNCTIONAL KIDNEY TEST	SPECIAL	CHEMICAL	EXAM. BY DR.	DATE RTD.
DATE CARD BEGAN: DATE CARD FILLED:		GRAND TOTALS								

Fig. 17.—Card (yellow) used for filing requests for special urine examinations.

A MODIFICATION OF THE TECHNIC OF THE VIVIDIFFUSION METHOD OF ABEL*

By H. C. van der Heyde and Withrow Morse, Morgantown, W. Va.

THE principal difficulty encountered in the vividiffusion method first suggested by Abel seems to be the trouble encountered in the preparation of the dialyzing tubes, as one may judge from the several suggestions which have been made to simplify this procedure.

In this laboratory, we find that by following the method outlined here, it is possible to save much time and patience.

A glass rod of a size slightly larger than the diameter of the tubes within the apparatus is dipped about a decimeter into a fairly liquid collodion and about this rod is wrapped lengthwise, a piece of fish bladder tissue about 12 cm. long and 1.5 cm. wide, permitting an extension of about two centimeters of the tissue beyond the end of the glass rod, so that one may remove the cylinder by pulling upon this extension. The cylinder is slipped off the rod before the collodion has set to such an extent that it will cause the tissue to adhere to the rod. The cylinders may then be dried by clamping them in a vertical position, or by pinning them to the sides of the table. If it is desired to limit the collodion as much as possible, the rod may be bathed with a somewhat more viscous collodion down one side and the tissue wrapped so that only the overlapping edge comes into contact with the collodion. It generally happens with this method, that one must apply a line of collodion down the seam to insure perfect adhesion.

Hess and McGuigan (Jour. Pharmacol. and Exper. Therap., 1914, vi, 1) modified Abel's original technic (Jour. Pharmacol. and Exper. Therap., 1914, iv, 611) and more recently, Love (Med. Rec. 1920, xcviii, 649) has advocated the use of chicken intestine for the purpose of replacing the collodion tubes. We are under the impression that the modification suggested above may make the application of this valuable method somewhat easier.

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EDITORIALS

Present-Day Methods for Studying the Problem of Ventilation

INVESTIGATION of the problem of ventilation is greatly hampered by the fact that of all animals man alone is concerned. This limits the possibilities for the investigation of the problem which, in brief, consists in comparing the general well being and comfort of man with the physical conditions of the indoor atmosphere in which he is living.

In a previous editorial in this journal,[1] the older theories of ventilation were reviewed and it was shown that neither the chemical composition of the air nor the presence in it of organic poisons has anything to do with the unhealthful effects which are associated with living in inadequately ventilated places. The conclusion was arrived at that it is the cooling effect of the air on the body which determines its healthfulness. The body is constantly producing heat by the metabolic processes which go on in it. This heat must be lost as quickly as it is produced, else will the temperature of the blood rise and the secondary reactions of a mild fever be the result. A great part of this heat loss occurs at the surface of the body by physical factors which depend on the cooling effect of the air. Of these factors radiation, convection and

evaporation are the most important although the last mentioned of these only comes into play to any considerable extent at the surface of the body when the air temperature is above a certain level which is somewhere about 70° F. There is, of course, some heat constantly lost by evaporation of water in the lungs but in man this is relatively constant (provided the absolute humidity and the temperature of the air remain unchanged), and it is the loss of heat from the skin by radiation and convection which is subject to alteration by changes in the blood flow in the superficial vessels. By observations on rabbits, N. B. Taylor and I have found by the use of thermocouples that there may be a difference of several degrees of temperature between the deeper tissues and those that immediately underlie the skin. This indicates a great cooling effect at the surface of the body. When the blood vessels in the subdermal tissues become dilated, and a large proportion of the blood as a consequence becomes drafted to the surface, this cooling effect may become greatly increased and when constriction of these vessels occurs it may become very slight because of the low heat conductivity of the skin and subcutaneous fat.

In poorly ventilated places the cooling influence of the air becomes reduced for several reasons: first, the temperature of the air rises, thus diminishing radiation from the skin (which is dependent upon the difference of temperature between skin and air), as well as reducing the heat which is lost in the air passages in warming the inspired air; second, the air stagnates so that there are only feeble convection currents; third, the humidity rises (both relative and absolute), so that less heat is lost both in the respiratory passages (in saturating the expired air with water at body temperature), and at the skin. To compensate for the lesser cooling, the heat-controlling centers cause more blood to be sent to the surface of the body, by vasodilation of the cutaneous vessels, so that the skin becomes flushed, and this may become so marked as to cause throbbing in the head and other symptoms. This drafting of blood to the surface causes less to flow in the vessels of the viscera so that the digestive and other glandular functions are hampered and the brain is inadequately supplied, leading to drowsiness, etc. The mucous membranes of the upper respiratory passages also become swollen and congested, due largely to capillary dilation, and therefore more susceptible to infections. But the attempts to keep down the temperature are not restricted to readjustments of blood supply, for there is also a cutting down of the body furnaces by depression of the metabolic functions, and it is quite likely that incompletely metabolized substances accumulate in the tissues and organs and clog the wheels of life.

Probably a most important effect of the inefficient cooling is dependent on the fact that the rather steep temperature gradient which we have seen to exist normally between the surface and the deeper tissues of the body now disappears, with the consequence that the thermic nerve endings in the skin are no longer stimulated. The stimulation of these nerves depends not on the actual temperature of the skin, but on differences in temperature between the skin and the deeper tissues so that when the surface of the skin is at about the same temperature as the deeper structures they are not excited. The impulses set up by the stimulation of these thermic nerve endings are important in

maintaining the tone of the nerve centers. They furnish, along with visual and acoustic sensations, the most important afferent impulses to the nerve centers, to keep them in a "wakeful" state.

The practical problem in ventilation is therefore to find simple methods by which the cooling influence of the atmosphere may be measured. An ordinary thermometer is of little value since it merely records the temperature of the glass and wood of which the instrument is made. A wet bulb thermometer is somewhat more valuable for its reading, by comparison with that of the dry bulb, measures the extent to which evaporation of moisture into the air is capable of lowering the temperature of the surface of the instrument. Until quite recently, however, these were the only two physical instruments that could be employed to measure the cooling influence of the air, and consequently the efficiency of ventilation. A much more reliable instrument is the Kata thermometer of Leonard Hill.[2] This is an alcohol thermometer reading between 95° and 100° F. It is first of all placed in water at about 105° F., and when the alcohol has expanded to the full extent for that temperature, the thermometer is withdrawn and the time required for the meniscus to fall from 100° F. to 95° F., is measured by a stop watch. The above range of temperatures being about that of the body, it is clear that the cooling of the instrument will be proportional to the cooling at the surface of the body. To calculate the amount of heat which is actually lost, it is necessary to express the cooling power in heat units per square centimeter of surface per second. The unit of heat is a calorie (i. e., the amount of heat necessary to raise the temperature of 1 gm. of water through 1° C.), but for the present purpose one thousandth of a calorie is taken; that is, a millicalorie. In order to convert the reading in seconds into millicalories per square centimeter per second, all that is necessary is to divide seconds by a factor (the Kata factor), which is written on the stem of the instrument. When used with its surface dry, the cooling of the instrument is dependent upon convection and radiation, but it can be made to include the influence of evaporation as well by keeping the surface moist by covering the bulb with muslin (muslin glove finger) kept moistened with water.

Numerous readings have been taken by the Kata thermometer under all varieties of atmospheric conditions, both without and within doors, and the most significant feature of the results is that in the former case the readings vary greatly within short periods of time whereas indoors they are usually more or less constant. Even in relatively stagnant humid air, the fluctuations outside are in general much greater than inside, even in a fairly well-ventilated room. Leonard Hill in his report for the Medical Research Committee,[2] publishes numerous charts to illustrate these differences and also to show that the readings by the wet and dry Kata thermometer are very much more sensitive than those taken simultaneously by the wet and dry bulb thermometer of the usual type. Now, obviously, the most ideal atmosphere for good health and comfort is out of doors, which indicates that in rooms we should endeavor to imitate the outside conditions with regard to cooling power as closely as possible. We should aim not only at air which can cool the surface of the body at a rate that is proportional to the rate of heat production, but we should imitate the outside variability in cooling power. Monotony of cooling conditions in a

room is unnatural and should be avoided if the inhabitants are to remain alert. On this account Hill is inclined to recommend for ordinary class rooms that they should be heated by low pressure steam pipes with open windows instead of being heated and ventilated by the plenum system. For larger meeting places, however, a plenum system with frequent inlets and outlets must be used.

It is, of course, admitted that observations on ventilation made in the British Isles with their relatively constant temperature throughout the year, are not directly applicable to the American continent with its extremes of heat and cold. Before the results can be made applicable, there must be independent observations on this continent, and the practical question in ventilation at present is to have a sufficient number of such observations made so that definite conclusions may be drawn. Much useful work in this direction has already been done by the New York Commission on Ventilation and in the public schools of Chicago, but much more work is required. In a general way the problem is to compare measurements made by physical instruments (Kata thermometer) of the cooling effect of the air in various parts of the hall or room with the well-being and comfort of the individuals that occupy it. It is with regard to the quantitative evaluation of comfort that the greatest difficulties arise, for, to make the comparisons of any value it is necessary to adopt certain more or less arbitrary standards. The New York Commission used various psychologic and physiologic tests and in school rooms the responsiveness and quickness of the pupils in answering questions have been useful, but for practical purposes it is probable that much useful information could be collected by merely asking the individuals their opinion of the ventilatory conditions and recording their impressions in general terms. By collecting large numbers of observations, errors of judgment would be ruled out.

With regard to the measurement of the cooling power, the ideal instrument to use is of course the Kata thermometer but there is one objection to it, namely, that it demands some time and care to obtain an adequate number of readings. If readings were to be taken at various parts of a hall, for example, it would necessitate observers at all these places. To make the instrument more practical Hill has therefore more recently devised Kata thermometers that are kept constantly heated by an electric heating device, the simplest form of these being the so-called "comfimeter." This consists of a metal tube which widens below to a cylindrical chamber near the upper end of which (an inch or so from the tube), is a metal partition carrying on its lower face a socket for an electric (carbon) lamp. Just above the partition the walls of the cylinder are provided with openings. The lamp heats the air in the cylinder above the partition and this causes cool air to enter by the openings and ascend the tube, and the temperature of this ascending column of air is measured by the thermometer which hangs in its center. It is clear that the temperature of this air, the heating power of the lamp remaining constant, will depend on the cooling influence of the atmosphere. We have made numerous comparisons between the readings by the comfimeter and those by the Kata thermometer and have found that they correspond with

entirely satisfactory closeness. By using several comfimeters throughout a class room we propose to compare the readings with the collective opinion of students sitting near, as to the ventilatory conditions. The instrument is so easy to use that it should be possible to have large numbers of observations recorded and thus perhaps to have at last a reliable method by which the efficiency of a system of ventilation could be tested.

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—*J. J. R. M.*

Experimental Influenza Bacillus Infection in Man

WITH the lapse of time since the 1918 influenza epidemic we find progressively less literature appearing on the subject. This is but natural. The storm having passed, we are inclined to forget it. Interest will lag more and more as time goes on until, with the advent of the next pandemic prevalence we will once again be taken unawares. If the interval be sufficiently long we will first pass through a period of conjecture as to what the strange new disease is, then one in which we suspect that it may be our old friend influenza, and finally after it has run a great part of its course we will decide that it is influenza, and will hasten to combat the pestilence with latest and most approved methods. The disease will, as in the past, pursue its course, little influenced by our frantic efforts.

During and after the next pandemic the literature will again be flooded with clinical description, with epidemiologic data, with immunologic observations, and with discussions pro and con, as to whether the disease is identical with that which swept the world in 1918.

In the case of influenza, we appear indisposed to profit by the experiences of past epidemics. Innumerable observations have been made since 1918, observations and theories which are believed by their sponsors to be entirely new and unprecedented. It was stated that influenza affected different age groups in 1918 from those chiefly stricken in 1889 and 1890. It has been stated that the students of the 1889 epidemic believed in an origin from an endemic focus in Turkestan. Now it is believed that the disease is widely distributed in endemic form during the interepidemic period. If one will but take the time to study the records following the 1889 epidemic, one will discover that the clinical and epidemiologic characteristics were the same, that the age incidence was the same, in short that the disease was true to type in its essential manifestations; also that the possible or even probable universal distribution of a virus of low virulence during the interepidemic period was fully discussed after the earlier pandemic. Even on the subject of bacteriologic etiology we are little farther advanced today than we were 25 or 30 years ago.

At that time it was quite generally conceded that the Pfeiffer bacillus was the cause of the disease. Yet there were many dissenters. Today we admit that we know nothing definitely of the causative agent but the discussion remains now as formerly, whether the influenza bacillus is or is not the cause of epidemic influenza.

If we are to profit by the disastrous experiences of the last epidemic; if we are to be better prepared to combat the ravages of influenza, it will be important that the disease be not completely forgotten during the next few years. Influenza and associated or similar conditions must be studied from all points of view, from that of the clinician, the bacteriologist, the immunologist and particularly the epidemiologist. Until the time when a specific vaccine, immune serum or other similar substance has been developed, greatest hope for the moderation of epidemic prevalences rests with the work of the epidemiologist. There are diseases, which like smallpox, have been brought under control, even though we know little regarding the causative germ. Incidentally we may expect considerable aid from the development of a vaccine directed against the secondary invaders.

It will be of considerable help if the rôle of the influenza bacillus can be fairly definitely established. There is some evidence indicating that this microorganism is not the primary cause of the disease. The evidence is, however, chiefly of a negative character. It has been claimed by many that the bacillus is of no pathogenic significance whatever. The work of Blake and Cecil, of Cecil and Steffen, and of Parker, has done much to establish the pathogenicity of the influenza bacillus.

Blake and Cecil innoculated the nose and throat of monkeys with a virulent culture of influenza bacillus and produced an acute upper respiratory tract infection which was quite similar to influenza as it occurs in man. The experimental infection was characterized by rapidity of onset, marked prostration, fever, leucopenia and a certain degree of contagiousness. Some of the cases developed secondary involvement of the lower respiratory tract. This work indicated that in virulent form the influenza bacillus is capable of producing an acute inflammation of the upper respiratory tract in monkeys. Parker demonstrated that a soluble toxin is formed in culture by the influenza bacillus, which is fatal on inoculation into rabbits.

Cecil and Steffen have continued the experiments of Blake and Cecil by attempting to transmit infection to man. They were able to produce an acute infection of the upper respiratory tract by the application of influenza bacillus cultures to the mucous membrane of the nose and throat. The recently isolated germ had been grown on chocolate blood broth medium and was used when the growth was six hours old. They likewise produced symptoms after the application to the upper respiratory mucosa of small amounts of peritoneal exudate from a monkey, dying from influenza bacillus peritonitis. Cultures passed through Berkefeld filters produced no symptoms whatever when applied in the same manner. The infection produced was characterized by the symptoms of acute coryza, with considerable prostration, and occasionally with headaches or other general pains. There was an associated leucopenia. As stated, these symptoms were not produced by filtrates and they were not

produced when the hemolytic streptococcus was substituted for the influenza bacillus. In the latter case, acute follicular tonsillitis, with leucocytosis, resulted. Pneumococcus cultures applied in the same way produced no symptoms and no elevation of temperature.

By their work Cecil and Steffen have shown on a small number of cases that the influenza bacillus in virulent form can produce an infection of the upper respiratory tract simulating in its clinical characteristics either acute coryza or a very mild influenza. None of the experimental cases developed an increased temperature, but they point out that the fatal case of influenza pneumonia, from which the virulent culture was isolated, also ran a very low temperature during the period under observation. They have also shown that the influenza bacillus when inoculated onto the mucous membrane usually disappears within a short time but that it may remain present for several days or even weeks after the inoculation.

The work of these various observers has shown that a virulent influenza bacillus can cause an infection of the upper respiratory tract in man. The organism may at times be quite highly invasive and may, under experimental conditions, at least, be a primary invader. It has not shown that the influenza bacillus is the cause of epidemic influenza. The work has carried our knowledge a step forward by demonstrating that at all events the influenza bacillus is not an organism to be lightly disregarded and that, no matter what its importance as a secondary invader in influenza, it must be regarded as a potential cause of primary acute infection of the upper respiratory tract.

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—*W. T. V.*

Silver Arsphenamine

FOR some time accounts have appeared in medical literature of a new arsenic preparation used in the treatment of syphilis. This has been called "silver salvarsan, or more precisely the sodium salt of silver-diaminodihydroxy arsenobenzene, and it contains approximately 22.5 per cent of arsenic and 14 per cent of silver. It is presumed that the silver for which spirochetes have an especial affinity serves as an anchor for the arsenic, and that therefore the drug, despite its lower arsenic content than arsphenamine, is more active therapeutically.

Animal experimentation seems to show that silver salvarsan is twice as effective as the old salvarsan (606) and three times as effective as neosalvarsan (914). Kolle states that silver salvarsan is old salvarsan in active form plus silver, and that 0.25 silver salvarsan is the equivalent of 0.4 of old salvarsan.

Dreyfus in discussing his experience with this drug in treatment of syphilis of the nervous system says that silver salvarsan is three times as efficacious as

the old salvarsan, that it acts more quickly, its toxic dose is higher, and it has the advantage of a combined silver and arsenic effect. He says that in nervous syphilis the indications are that the new preparation promises to be more valuable than the older ones, even when the latter are used in conjunction with mercury. It is Dreyfus' experience that, especially in early cases, both subjective and objective symptoms show marked signs of retrogression within two weeks. More care is necessary in tabes than in other conditions, but in all, small doses with carefully graduated increases are used.

Boas and Kissmeyer, after treating 62 cases representing all stages of syphilis, and using mercury with it, found that silver salvarsan was just as effective as old salvarsan, and in addition it was more soluble and easier to handle. They prefer it for these reasons only. Körsbjerg on the other hand is very enthusiastic over the new drug and has been so impressed by its effects that neither he, nor his chief Jersild, use mercury either as an accompaniment nor as a follow up treatment. There were 32 cases in the series reported, of which 19 were secondary. In every case all symptoms had vanished within two weeks of the first injection.

The use of this silver-arsenic preparation seems from the reports to be attended by more danger than the older preparation. This danger is reflected in the dosages used, i. e., from 0.02 to a maximum of 0.25, in dilute solution. Anaphylactoid symptoms,—redness and swelling of the face and buccal mucous membrane,—pyrexia; cutaneous eruptions which are usually transient, and occasionally severe dermatitis; syncope, collapse, vomiting, vertigo and headache; and icterus are all listed as secondary effects.

Neurorecurrences seem from reports to be fewer after silver salvarsan than after the older drugs, but the cases are too few to be decisive.

A large series of cases treated with silver salvarsan is that of Behring, who reports upon his experience in giving 3,200 injections in 259 cases. In this series icterus occurred 9 times, and angioneurotic symptoms 6 times. There was one death. In his experience no venous thrombosis occurred. Eruptions and icterus were not more commonly observed after silver salvarsan than after the older preparations. Silver salvarsan is well adapted to the abortive treatment of syphilis. Behring says that a definite verdict cannot as yet be given with reference to tabes and central syphilis, although tabes is apparently favorably influenced.

Wiener says that the curative effects in primary lesions, secondary and tertiary symptoms are very favorable and apparently not inferior to the results of a combined course of neosalvarsan and mercury in the customary dose.

The most recent report on this silver-arsphenamine is based upon the experience of Major Walson of the Medical Corps, U. S. A., and has to do with the treatment of 800 patients, and more than 6000 injections. The method of treatment recommended by the Board of Medical Officers, and the method used by Walson was as follows:

An interval of seven days between each dose in each course of treatment. Treatment to consist of four courses of silver salvarsan and gray oil.

In the first course of treatment the first dose to be fifteen hundredths

(0.15) gm. of the drug. The second dose to the two tenths (0.2) gm., and each of the remaining five doses of the course to be three tenths (0.3) gm. of the drug.

At the end of the first course of treatment a Wassermann blood test is made and then thirty days' rest.

In the second course of treatment three-tenths (0.3) gm. of the drug is given at each of seven injections, at seven day intervals, and is followed by two and one-half months' rest.

The third and fourth courses are the same as the second, with ninety days' interval between the two. Gray oil is used in conjunction with and at the same time as each injection of silver salvarsan, using eight hundredths (0.08) gm., by intramuscular injection.

A blood Wassermann is recommended after each course and a spinal fluid Wassermann after the second.

Such is the army intensive treatment. As against it Hoffmann says that one complete intensive course of treatment with mercury and silver salvarsan in primary syphilis will give a complete cure. Hoffmann, Neisser and Scholz believe that if treatment can be commenced in the prepositive Wassermann stage the disease can be cured in from 80-100 per cent of the cases. Nevertheless it is to be borne in mind that all persons who have had syphilis, no matter how rapidly the clinical signs disappear, nor how soon the Wassermann reaction becomes negative, should be watched both for clinical and serologic recurrences.

It seems from a review of the literature, that in silver-arsphenamine we have a more potent antispirocheticide than any heretofore in use, and one which should be used with the greatest care. It seems to represent a real therapeutic advance.

REFERENCES

Walson: Amer. Jour. Med. Sc., 1921, clxi, 418. Other quotations are made from the reviews in *Medical Science; Abstracts and Reviews;* and in the *American Journal of Syphilis.*

—*P. G. W.*

International Organization and Public Health

BUCHANAN[1] discusses the League of Nations and Public Health. It is evident that, while all medical science, both preventive and curative, is international and free for any one who chooses to use it, still a League of Nations might do many things in order to improve the living conditions of the populations of its constituent members. If all the nations of the earth, or even if the great nations of the earth, should combine in efforts to stamp out infectious diseases, great good could be accomplished. Since 1903 there has been an agreement among the great nations to notify all others when certain infectious diseases, notably the plague and Asiatic cholera, occur in any one country. Too frequently these notifications pass through diplomatic channels

[1]Lancet, London, 1921, cc, 415.

and, like all procedures depending upon red tape, are slow in their dissemination. Even the war left untouched, largely at least, one international prerogative, and that is, the right of any nation to protect its people from the importation of infection. For centuries there has been more or less conflict in regard to quarantine laws between the protection of health on one side and the interference with commerce on the other. An example of how improvement might be brought about is at hand in the distribution of anthrax quite broadly over the world from Japan in recent years through shaving brushes. Several countries have found it necessary to take action in this matter. If Japan would guarantee the thorough sterilization of its exported articles, there would be no danger of carrying infection to other countries, and at the same time there would be no necessity for other countries to exclude certain importations from Japan. Recently, the British Government has been obliged to prohibit the importation of shaving brushes from Japan. The United States has, we believe, not been quite so radical in dealing with the Japanese, but has demanded that all shaving brushes brought from Japan into this country should be sterilized. That this has not absolutely stopped the importation of anthrax-laden brushes is shown by the fact that now and then infection through this agency is reported. Each government must rely upon its own consular service, largely at least, in protecting itself from infection from abroad. When we realize that consuls, as a rule at least, have no knowledge of health matters and often no interest in them, it is easy to see the inadequacy of this provision. The International Red Cross has taken up its residence in Geneva, has built certain laboratories, is collecting a library, and is publishing in several languages, a fairly creditable journal. This work, however, is voluntary, is dependent upon uncertain support, and up to the present time at least, has no common or universal sanction. Buchanan states that the League of Red Cross Societies has already found itself hampered by the absence of a health section at the League of Nations. Since 1907 there has been a semi-official International Health Publication issued in Paris. This was established by the convention held at Rome. This central office has been of great value, and it would be unfortunate if it should not be chosen as the nucleus of a greater International Health organization.

Much might be accomplished by the internationalization of vital statistics. The basis on which these are collected in the different countries is widely variable and one must be careful in drawing conclusions from statistical data secured in this way; in fact, there is no country in the world where vital statistics, even concerning births and deaths, are rigidly correct. The best we can say is that in some countries they are approximately correct. Even in the same country, the nomenclature of disease changes from time to time and in more than one instance this has deceived medical writers who have concluded that, because a given name no longer appears in the mortality statistics, the disease which it represents has ceased.

—*V. C. V.*